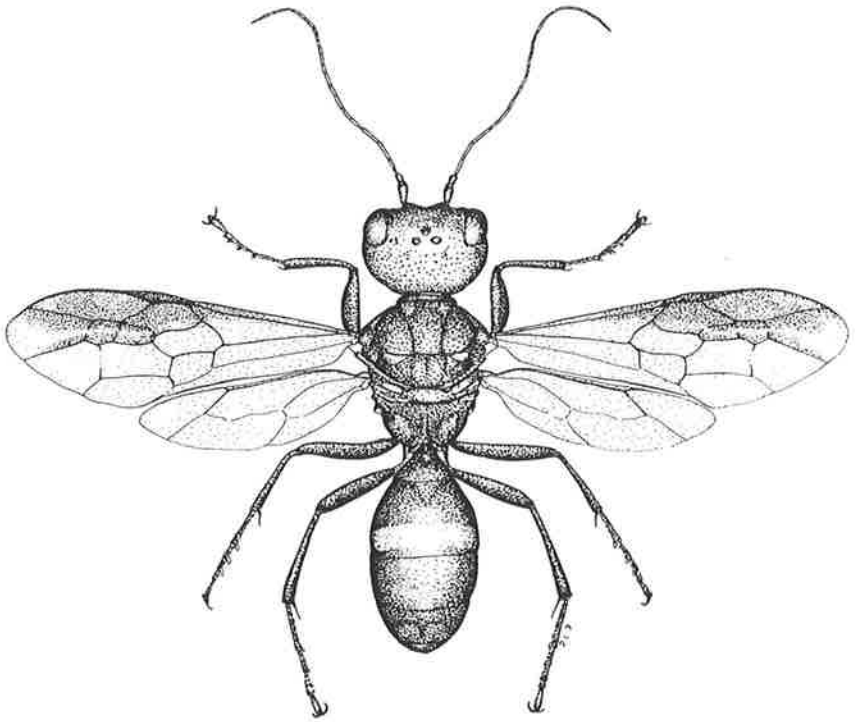
The biology of the parasitic wasp *Taeniogonalos venatoria* Riek (Trigonalyidae) and its *Eucalyptus*-defoliating host *Perga dorsalis* Leach (Hymenoptera: Pergidae).



by

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Summary

The biology and behaviour of *T. venatoria* and *P. dorsalis* were studied in the Adelaide region between 1987 and 1990.

A multivariate study of the distribution of larvae of *Perga dorsalis* was undertaken to determine the properties of the sites in which the larvae were found. They were found (a) in areas with 500 to 1000 mm of annual rainfall, (b) on *Eucalyptus camaldulensis, E. leucoxylon* and *E. torquata* (as well as on several rarer species), (c) most frequently on young trees, and (d) most frequently on trees which were planted in unnatural surroundings (such as by the roadside or in gardens). Parasitised larvae were collected from areas of high larval incidence, and *T. venatoria* were reared from them.

T. venatoria and other trigonalyids have an ovipositional strategy which is unique among the Hymenoptera; the adult female oviposits on foliage, and successful parasitisation of the host, in this case *P. dorsalis*, is dependent upon the folivorous *P. dorsalis* larvae consuming the parasitoid's eggs together with the foliage.

Laboratory experiments indicated that eclosion in the host's gut is dependent upon the chorion first being broken by the mandibular action of the host, and then being exposed to an acidic solution. Dissections of host larvae indicated that the first instar larva penetrates the host's gut to enter the haemocoel, but then does not continue development until the host is about to pupate. Five larval instars were differentiated on the basis of mandibular size; the first three instars are endoparasitic, and the last two ectoparasitic.

The mean fecundity of *T. venatoria* was much higher than that recorded for other hymenopterous parasitoids; egg counts on foliage and in dissected females indicated that egg development continues during the lifetime of the female, and that total egg-counts can reach 10,000 per female. The mean longevity of females in the laboratory was 3 days, but increased to 6 days when honey was provided.

Reproduction is almost invariably parthenogenetic. The male of *T. venatoria* is very rare, with less than one male in a thousand being reared in this study. The male is described here for the first time.

T. venatoria is univoltive, and adults emerge in autumn (March to April). Oviposition by adult females occurs shortly after the eclosion of first instar *P. dorsalis* larvae. The latter feed over winter, and enter the soil in early summer, when they diapause.

Although trigonalyids are generally considered to be obligate hyperparasitoids, *T. venatoria* was reared to adulthood both as a primary parasitoid and as a hyperparasitoid. This is the first experimental demonstration of the ability of any trigonalyid to act both as a primary parasitoid and as a facultative hyperparasitoid. The evolutionary implications of this strategy are discussed.

The host-selection behaviour of *T. venatoria* was investigated in the field, and in the laboratory with olfactometers and a wind tunnel. Oviposition rates were also recorded on a variety of foliage types. As the response was negative to all chemical cues that were investigated, it was not possible to conclude that adult *T. venatoria* used chemical cues in either host-habitat location or in the acceptance of oviposition substrates. It is suggested, instead, that *T. venatoria* oviposits randomly on any foliage which its ovipositor is physically capable of penetrating.

Various biological and behavioural characteristics of *P. dorsalis* were also investigated to determine their possible effect on parasitisation by *T. venatoria*. One of these characteristics was diapause. *P. dorsalis* was shown to undergo prolonged diapause, as has been shown previously for the closely related *P. affinis*. In individuals undergoing prolonged diapause, emergence did not occur in the autumn following soil entry, but was delayed for up to 4 years. *T. venatoria* also underwent prolonged diapause, within its diapausing host, and resumed morphogenesis only after the host pupated. The factors that break diapause in *P. dorsalis* are therefore ultimately those that also lead to the emergence of *T. venatoria*. Temperature and host larval size were found to be the major determinants of the length of time the host-parasitoid complex spent in diapause, whereas soil moisture affected emergence through its influence on the mortality of cocooned stages.

The second characteristic of *P. dorsalis* larvae that was studied was the 'leadership behaviour' demonstrated by some larvae. In colonies of

the gregariously feeding larvae, individuals were labelled with different colours of oil paint. By recording the changes in position of the larvae in some colonies, and by experimentally manipulating the positions of larvae in others, colonies were shown to contain a subgroup of larvae which were behaviourally more active. These larvae appeared to 'lead' colonies on their nightly foraging expeditions. It is argued here that these more active larvae, although more exposed to parasitoids such as tachinids, do not suffer a greater rate of parasitisation from *T. venatoria*. The colonies also demonstrated a circular defense strategy (cycloalexy), wherein the larvae form a 'heads-out' circle, and regurgitate sequestered *Eucalyptus* oils at parasitoids. *T. venatoria*, however, remains unaffected by this defense mechanism, as it does not require host contact for oviposition. The advantages and disadvantages of these behaviours are discussed in the context of their effect on the host-parasitoid relationship.

Finally, 'petiole chewing' was observed in the larvae of *P. dorsalis,* and an experiment was conducted to test the effect of this behaviour on larval weight. Petiole chewing is a behaviour in which the last larva in a colony leaving a consumed leaf severs the petiole of that leaf, thereby removing leaf remains and the midrib from the branch. *P. dorsalis* larvae which were prevented from petiole chewing gained more weight than did control larvae, suggesting that the effect of petiole chewing is not to 'sabotage' the putative host-tree defences which may be induced by leaf remains if left *in situ.* It is suggested that petiole chewing results in the removal of physicochemical cues used by parasitoids in host location, although parasitism by *T. venatoria* may again be unaffected by this behaviour.

The results of this study are discussed in relation to general hypotheses proposed for host selection by parasitoids. Possible evolutionary scenarios are presented to account for the observed behaviours of both *T. venatoria* and *P. dorsalis*.

Declaration

This thesis contains no material which has been submitted for the award of any other degree or diploma in any University, and contains no material previously published or written by another person, except where duly acknowledged in the text of the thesis.

This thesis may be made available for loan or photocopying, provided that acknowledgement is made of any reference to work therein.

Two published papers (Chapter 8 and Appendix 1) are included as part of this thesis. The species catalogue to world Trigonalyidae (Appendix 1) was compiled with A.D. Austin, but all other work in these publications is my own.

April 1992 Philip Weinstein.

Acknowledgements

Firstly and foremost I wish to thank my supervisors Andy Austin and Derek Maelzer. Andy's guidance and rigour have been invaluable, dating right back to his help in selecting a research project which has been most interesting. Derek has not only helped me to develop the ecological aspects of the project, but has also taught me how to think; for this and for his friendship I will always be gratefull.

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INTRODUCTION

Introduction

The Trigonalyidae is a small family of wasps that is unique among parasitic Hymenoptera in that they oviposit onto foliage and depend upon a folivorous primary host to ingest their eggs with plant material. Tachinid flies (e.g. Goniinae) are the only other insect group known to employ this mode of host infection (Askew, 1971). Some Chalcidoidea (e.g. Eucharitidae) also oviposit onto foliage, but they possess a planidial first instar larva which locates the host (Boucek, 1988), and their eggs do not eclose in the host's gut, as is the case for the Trigonalyidae. Members of the family are thought to be hyperparasitoids, attacking ichneumonids, tachinids, vespids or eumenids associated with phytophagous primary hosts, mostly sawfly or lepidopteran larvae (Clausen, 1940; Gauld and Bolton, 1988). However, there is a report of an Australian species, *Taeniogonalos maculata* (Smith), being a primary endoparasitoid of a pergid sawfly, but this is based on somewhat circumstantial evidence (Raff, 1934).

Apart from these unusual biological features, trigonalyids also possess a unique array of morphological characters. The shape of the head and mouthparts, presence of tarsal plantulae and relatively complete wing venation are archiac characters similar to those found in the Symphyta, while the presence of a propodeum, trochantelli and parasitic habits clearly place trigonalyids within the terebrant superfamilies of the Apocrita. However, the general body shape and terminal ovipositor are reminiscent of aculeate wasps (Wheeler, 1928; Yamane, 1973). The paraglossae are so archaic as to have caused the Trigonalyidae to be classified, at one stage, in a separate division of the Hymenoptera; the "Archiglossata" of Börner (1919). Their wing venation is considered to be possibly the most primitive within the Apocrita (Naumann, 1991), and this has led to suggestions

that the family represents a "missing link" between the Symphyta and Apocrita (Malyshev, 1968). There is no evidence for Malyshev's hypothesis that the Trigonalyidae arose from inquilines associated with gall-forming sawflies, and recent morphological studies have allied the family to the Evanioidea, Ceraphronoidea and Megalyroidea (see Rasnitsyn, 1980, 1988; Gibson, 1985; Johnson, 1988). The trigonalyids are therefore not considered to be in any way basal in the phylogeny of parasitoids, and their oviposition behaviour must therefore be a derived character.

Despite these exceptional morphological and biological features, trigonalyids have been little studied, possibly because of their sporadic occurrence and short-lived adult stage. The only major taxonomic works are those of Schulz (1907) and Bischoff (1938). The biology of trigonalyids has not been reviewed since Clausen (1940), and there have been few biological studies since then, despite the fact that some trigonalyids show potential as biological control agents (Carne 1969).

The present study was carried out in order to document for the first time the biology and behaviour of *Taeniogonalos venatoria* Riek, an Australian trigonalyid whose main host is the *Eucalyptus*-defoliating sawfly *Perga dorsalis* Leach. The biology of the latter species was also studied in order to establish in what ways its biology and behaviour might influence the effectiveness of *T. venatoria* as a parasitoid. As the hardwood industry in Australia expands, and as insecticide use becomes less acceptable, it is important to document the biology of potential biological control agents such as *T. venatoria*.

CHAPTER 1

THE TAXONOMY OF *TAENIOGONALOS VENATORIA* RIEK AND *PERGA DORSALIS* LEACH.

CHAPTER 1

The taxonmony of *Taeniogonalos venatoria* Riek and *Perga dorsalis* Leach

1.1 Introduction

In order to precisely define the identity of both the host and parasitoid whose biology and behaviour were studied during this project, the taxonomy of *Taeniogonalos venatoria* Riek and *Perga dorsalis* Leach were reviewed.

The need for accurate identification was reinforced by:

(1) The lack of published records of the occurrence of either *T. venatoria* or *P. dorsalis* in South Australia, and

(2) The collection in this study of male *T. venatoria*, this sex being previously undescribed for the species.

In addition to employing the identification keys available for both species (Benson, 1939; Riek, 1954, 1961, 1962a), locally collected specimens were compared to identified material in the Australian National Insect Collection (ANIC), using types where possible. For completeness, the male of *T. venatoria* is described here.

1.2 *Taeniogonalos venatoria* Riek

Taeniogonalos venatoria Riek is one of 12 described species and subspecies of the genus *Taeniogonalos.* Seven species and subspecies are described from the eastern half of Australia; none are known from Western Australia. The remaining 5 species and subspecies are described from Mexico, Brazil and Taiwan (Weinstein and Austin, 1991), possibly suggesting an expanded Gondwanian or circumtropical distribution for the genus. It is important to note, however, that many trigonalyid genera are poorly defined, and that several genera may infact not be monophyletic (Weinstein and Austin, 1991).

T. venatoria was described by Riek (1962a), after previously having revised the genus for Australia (Riek, 1954). The female can be distinguished from other species of Australian *Taeniogonalos* by the lack of a pronounced process on the 3rd gastral sternite, and by having 23-24 antennal flagellomeres (see Riek, 1962a for key to species). Previously, the species was known from inland N.S.W., Victoria and the A.C.T. (specimens in ANIC; Riek, 1962a). The present study records *T. venatoria* from Adelaide and the southeast of South Australia for the first time, thereby extending its known distribution considerably westwards.

Particular collection sites in South Australia are recorded in Chapter 2, where the habitat preferences of *T. venatoria* are also inferred from the distribution of its major host, *Perga dorsalis* Leach.

Eight male *T. venatoria* were reared during this study, and the male is described here for the first time. *T. venatoria* was previously known only from female specimens, except for one male which was reported, but never described, by Carne (1969). The latter male specimen was reared from the larvae of *P. affinis* Kirby collected in the Brindabella Ranges, A.C.T., and is currently held in the ANIC. The males reared in this study were all fom larvae collected in the Naracoorte region in southeastern South Australia, and they can be distinguished from other species of *Taeniogonalos* as follows:

Taeniogonalos venatoria Riek, 1962a : 92-95; Weinstein and Austin, 1991 : 417.

Description (supplementary to Riek, 1962a for the female):

Male:

Length: 9.0 mm (± 0.5mm, N=6) (female, though variable, averages 13mm); black, with yellow markings as for female.

Head: as for female. Antenna with scape, pedicel and 23 flagellomeres; tyloids on flagellomeres 6-14.

Thorax: as for female. Wingspan 16mm (± 1mm, N =6) (female, though variable, averages 24mm).

Gaster: smoothly rounded, less curved than in female; sternal projections absent (Figure 1.1); coloured band which encircles distal part of segment 2 interrupted mid-sternally by black concavity (in female this coloured band most usually extends to include the sternal projection).

Specimens examined: 6 males, 50+ females. Males reared ex pupae of *P. dorsalis* Leach, March 1989; parasitised larvae collected Naracoorte, South Australia, September 1988, P. Weinstein. Females reared ex pupae of *P. dorsalis* Leach, March and April 1988 and1989; parasitised larvae collected Adelaide region and Naracoorte, South Australia, September and October 1987 and 1988, P. Weinstein. All material in the Waite Institute collection, University of Adelaide.

Males of other Australian species are known only for *T. maculata* (Smith) and *T. tricolour* Rayment. In these species, the tyloids are more restricted than in *T. venatoria*, and are found on flagellomeres 7 to 12 or 13. In both males and females, the antennae and legs of *T. maculata* and *T. triclour* are lighter in colour than are those of *T. venatoria*, in that they are brown rather than black.

In reviewing the taxonomic and biological literature on trigonalyids, information was collected for the family an a worldwide basis. Species descriptions published since the Trigonalyidae were last catalogued (Bischoff, 1938), and information obtained from the world's leading collections, were used to produce an up-to-date

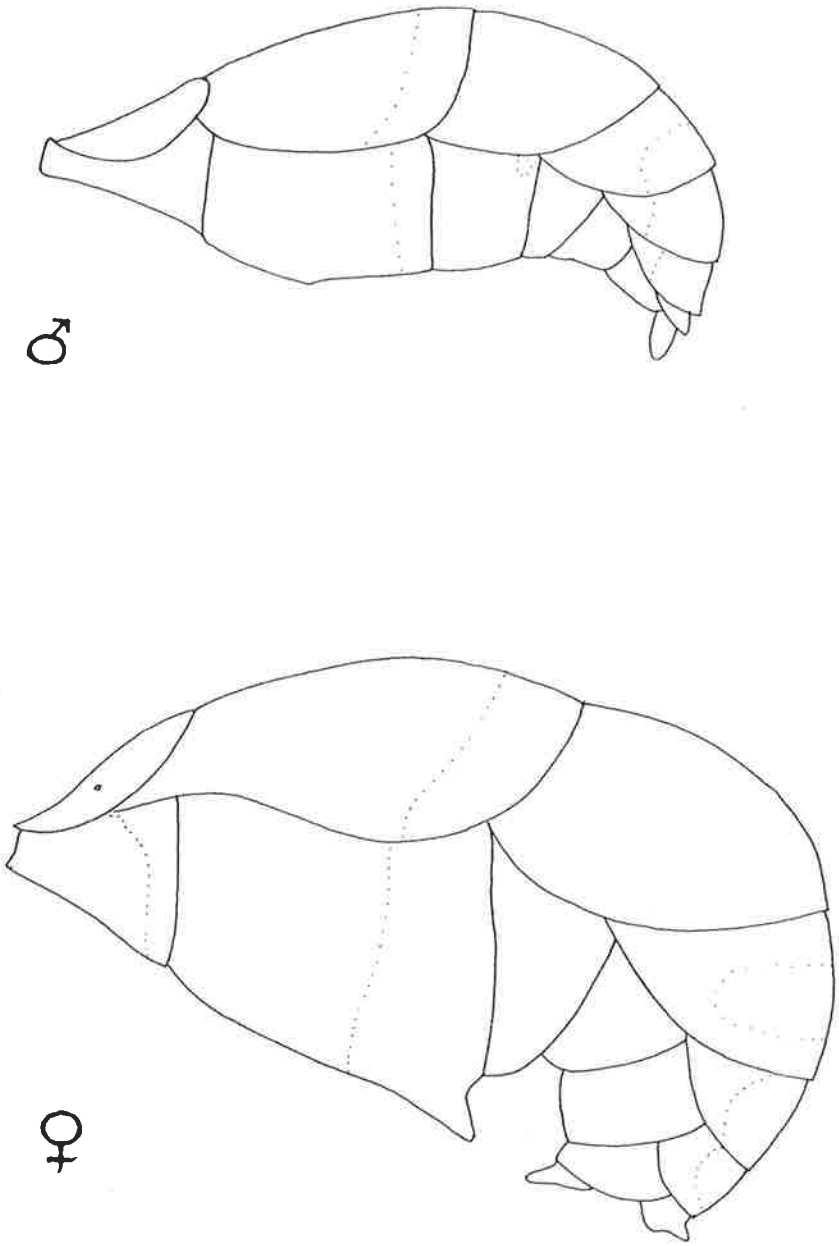


Figure 1.1
The gasters of a female and a male *T. venatoria,* illustrating the absence of a sternal projection in the male. 1cm = 0.5mm. Illustration by P. Dangerfield.

catalogue to species as well as to present some comments on the systematics and biogeography of the family; these are included here as Appendix 1.

1.3 *Perga dorsalis* Leach

Perga dorsalis Leach is the type species of the genus and was described from a male specimen collected in New South Wales (Leach, 1817). In revising the genus more than a centuary later, Benson (1939) experienced considerable difficulty in associating the correct female with the holotype, given the apparent similarity of several species, as well as the existence of other species described from males. Riek (1961) clarified the taxonomy of the species by redescribing *P. dorsalis* and the closely related *P. affinis,* in addition to describing several new subspecies for each. The taxonomy of other *Perga* species (*sensu lato*) unfortunately remains imprecise, and some individuals of other species show characteristics intermediate between those used by Benson (1939) to differentiate both species and genera. Given that *Perga* (s.l.) is a uniquely Australian genus with overseas relatives only at the subfamily level in South America, and that *Eucalyptus* defoliating Pergidae are of increasing economic significance, it is surprising that the generic and specific classification of the group has not been reviewed since Benson (1939).

The species of *Perga* dealt with in this study has a green-black iridescent gaster, an ill-defined oblique sulcus on the pronotal lobes, densely pilose lower genae, and hind femora which are in part darkly coloured. These characteristics clearly identify the species as *Perga dorsalis dorsalis* Leach (see Riek, 1961 for key). Previously recorded from N.S.W., Victoria and the A.C.T., the occurrence of this species near Adelaide and in the southeast of South Australia extends its known range considerably westwards. Precise collection sites and habitat preferences of *P. dorsalis* are given in Chapter 2.

CHAPTER 2

THE DISTRIBUTION AND HOST-TREE PREFERENCE OF *PERGA DORSALIS AND TAENIOGONALOS VENATORIA* IN SOUTH AUSTRALIA.

CHAPTER 2

The distribution and host-tree preference of *P. dorsalis* and *T. venatoria* in South Australia.

2.1 Introduction

The surveys described in this chapter were carried out in an attempt to quantitively define the preferred habitat of *P. dorsalis* and *T. venatoria* in South Australia. It was necessary to define this habitat in order to undertake further studies of the biology and behaviour of *T. venatoria*. Firstly, adult *T. venatoria* were to be reared from *P. dorsalis* larvae which had been located and collected in areas of high incidence, and secondly, the habitat characteristics could offer clues as to which factors may be significant in the host selection behaviour of *T. venatoria*. Carne (1965, 1969) showed that the preferred host trees of the related *Perga affinis* Kirby in N.S.W. were small *Eucalyptus melliodora, E. blakelyi* and *E. camaldulensis,* particularly in regions with an annual rainfall between 450 and 700 mm (18 and 28 inches). Rainfall, tree species and tree height were therefore all included in the present survey. Because the activities of man can potentially produce changes in the 'quality' of host trees, either by means of 'stress' or through altered 'apparency', an assessment was also made of the extent to which trees could be considered to be growing 'naturally'.

2.2 Materials and Methods

2.2.1 Distribution of *P. dorsalis* and *T. venatoria* in relation to rainfall.

Between 1987 and 1990, the presence or absence of *P. dorsalis* was recorded at 77 sites in the central districts and lower south-east of South Australia. These sites were selected to lie in as wide a range of isohyets as could be feasibly visited, and are marked in Figure 2.1. At each site, 3 subsamples were taken to locate larvae (or adults) of *P. dorsalis.* For each subsample, an area of up to approximately 4km^2 was selected and carefully searched for 'suitable' trees, which were, on the basis of previous field experience, subjectively deemed to be likely to harbour *P. dorsalis.* These trees were predominantly small or regrowing *E. camaldulensis* and *E. leucoxylon*, preferably unnaturally exposed or watered (for example by the road side or in gardens).

In each subsample, all suitable trees were searched for larvae of *P.dorsalis.* If larvae were found in the subsample within 30 minutes, they were simply recorded as present. If larvae were not located within 30 minutes of searching they were recorded as absent. The next subsample at a site was taken in the next accessible area encountered which was similarly deemed likely to contain larvae. Subsamples were often more than 2 km, but always less than 6 km, from the site of the previous subsample.

At those sites that were visited in late March or in April, a search was also made for adult *T. venatoria* (see Section 3.4.3, Emergence phenology) and they were recorded if present. Adult *T. venatoria* were also reared from sawflies collected at a number of the sites (and kept subsequently on clean foliage); *T. venatoria* was recorded as "present" at a site if it was either seen or reared. The presence of both *P. dorsalis* and *T. venatoria* was plotted on a map of South Australia (Figure 2.1.), and the effect of rainfall upon the presence of *P. dorsalis* was analysed by single factor ANOVA and multiple range testing.

2.2.2 Incidence of *P. dorsalis* on various eucalypts.

A further, multivariate study of the distribution of larvae of *P. dorsalis* was undertaken during 1988 and 1989. Eucalypts from a number of areas were sampled at random to determine the species, height and status of trees upon which *P. dorsalis* occurred most frequently. The samples were taken between late August and late October, when large larvae are most easily spotted in partially defoliated trees (see Section 3.4.3, Emergence phenology).

Two areas were selected for sampling; Adelaide and suburbs, and the lower south-east of South Australia (Mt. Gambier, Penola and Naracoorte). Both regions have between 500 and 800 mm of rainfall per annum (see Fig. 2.1). It was assumed that the incidence of *P. dorsalis* was not influenced by rainfall between these limits, an assumption validated by the analysis of the *P. dorsalis* distribution by rainfall (see 2.3.1 Results).

The Adelaide region was selected because of the large number of unnaturally exposed or irrigated trees it contains, such as those in gardens and by the road side. Sample sites were selected at random from the 30 central maps of the UBD Adelaide Street Directory (25th edition, Map numbers 16-21, 24-29 and 32-49; Universal Press, 1987). Random numbers were used to (i) select a map, and (ii) select x and y coordinates upon a grid pattern with 0.5cm x 0.5cm squares, which was superimposed upon each map. Each sample site thereby selected was 111m x 111m, and all *Eucalyptus* trees within it were examined. The following data were recorded for each *Eucalyptus* tree: species; height (<2m, 2-6m, >6m); distance from road (<5m, >5m); any indication of exposure to unnatural pruning, irrigation or shading; and whether or not *P. dorsalis* larvae were present. Trees were scored as 'manipulated' if they were less than 5 m from the roadside, or if they were pruned, irrigated or shaded significantly by buildings; otherwise they were scored as 'natural'.

The lower south east was selected because of the presence of many remnant stands of natural *Eucalyptus* woodland, containing known host species for *P. dorsalis* (*E. camaldulensis, E. leucoxylon*). Sample sites were selected at random from subsections of 1:100,000 topographic maps of Mt. Gambier, Penola and Naracoorte (Natmap, Edition 1). The

subsections were of 240, 770 and 475 km² respectively, with the following grid references: Gambier 7022/650200 to 750200 and 650440 to 750440; Penola 7023/760500 to 970500 and 760870 to 970870; Naracoorte 7024/550270 to 750270 and 550520 to 750520. Within these subsections, random numbers were used to select x and y coordinates upon the existing grid pattern of 1cm x 1cm squares, giving sites of 1km x 1km. At each site, the first 111m x 111m area accessible by road was sampled. For each *Eucalyptus* tree present, species, height, manipulation, and presence of *P. dorsalis* larvae were recorded, as for the Adelaide Region. When necessary, trees were carefully searched with binoculars.

Data were recorded for a total of 2348 *Eucalyptus* trees from 72 randomly selected sample sites. Three-dimensional contingency table analysis (Zar, 1984; Tabachnick and Fidell, 1989) was used to determine whether there was any influence of species and height of tree on the presence of *P. dorsalis*.

2.3 Results

2.3.1 Distribution of *P. dorsalis* and *T. venatoria* in relation to rainfall.

Each site of potential distribution is listed in Appendix 2.1, and the local annual rainfall is given, together with the presence or absence of *P. dorsalis* in each of the three subsamples taken at that site. Those sites at which *T. venatoria* was recorded are also given. The information is summarised on the map of the central districts of South Australia (Figure 2.1), where the shaded area corresponds to the approximate (interpolated) distribution of *P. dorsalis* and *T. venatoria.* The modal rainfall for the presence of *P. dorsalis* was 700 mm, and there were very few positive records for either *P. dorsalis* or *T. venatoria* in areas with an annual rainfall of less than 500 mm or more than 1000 mm.

In Table 2.1, the following data are given for each of 9 classes of rainfall: annual rainfall in mm; total number of samples taken; number of samples in which *P. dorsalis* was found and the mean percentage of samples in which *P. dorsalis* was found. The mean percentages of samples in which *P. dorsalis* was found differed significantly between categories of rainfall (ANOVA, $F=8.475$, $p=0.0001$; Appendix 2.2). The means in each category were compared by Fisher PLSD, and the significant differences in means are indicated by bars in Table 2.1. The means for the 500-600, 600-700, 700-800 and 800-1000mm classes did not differ significantly from each other, but they were significantly higher than the means for the remaining classes. The means for the remaining classes did not differ significantly from each other. The results indicate that *P. dorsalis* (and *T. venatoria*) occurred more frequently in areas with an average annual rainfall of between 500 and 1000 mm.

2.3.2 Incidence of *P. dorsalis* on various eucalypts.

A total of 2348 eucalypts were examined, and 1510 of these belonged to 7 species upon which *P. dorsalis* larvae were feeding. These species were *E. camaldulensis, leucoxylon, torquata, gomphocephala, sideroxylon, porosa* and *diversifolia.* Species of *Eucalyptus* upon

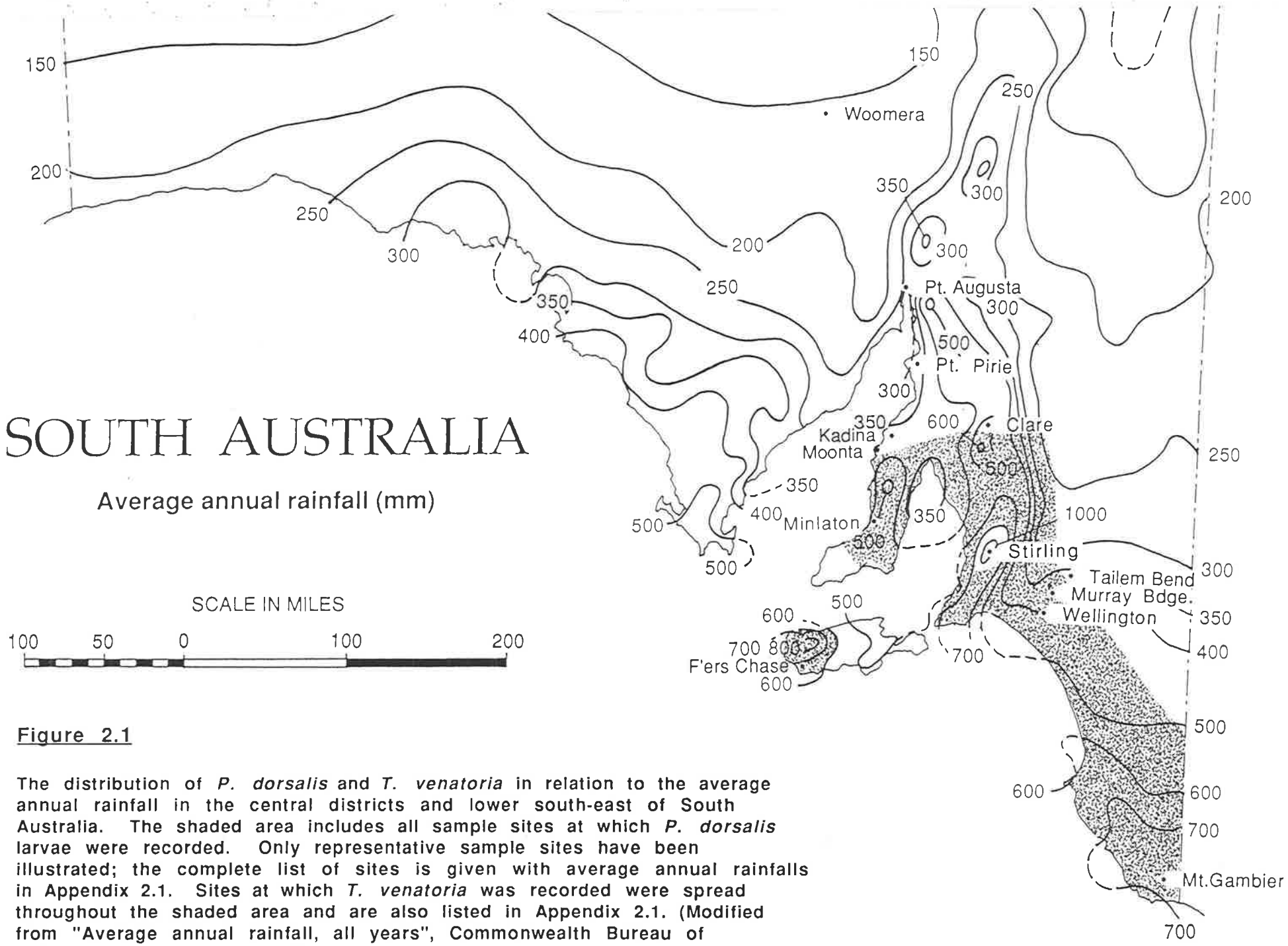


Figure 2.1

The distribution of *P. dorsalis* and *T. venatoria* in relation to the average annual rainfall in the central districts and lower south-east of South Australia. The shaded area includes all sample sites at which *P. dorsalis* larvae were recorded. Only representative sample sites have been illustrated; the complete list of sites is given with average annual rainfalls in Appendix 2.1. Sites at which *T. venatoria* was recorded were spread throughout the shaded area and are also listed in Appendix 2.1. (Modified from "Average annual rainfall, all years", Commonwealth Bureau of Meterology, Melbourne 1968).

Table 2.1

The average annual rainfall in each of 9 classes of rainfall in which the presence or absence of P. dorsalis larvae was recorded, the number of samples, and the mean percentage of samples in which P. dorsalis larvae were detected.

* The bars indicate which means differ significantly from each other at the 95% level, by Fisher PLSD testing.

Rainfall (mm)	No. of samples	No. of samples with larvae	Mean % samples with larvae	Sig. diff's.
>1200	3	0	0.00%	
1000-1200	6	1	16.70%	
800-1000	21	13	61.90%	
700-800	21	15	71.40%	*
600-700	42	30	71.40%	
500-600	45	26	57.80%	
400-500	56	16	28.60%	
350-400	22	2	9.10%	
<350	15	1	6.70%	

which *P. dorsalis* larvae were not found were not all identified, but included many stringybark eucalypts. *E. macrorhyncha, microcarpa, erythrocorys* and *viminalis* are additional host trees which were noted during the survey, but which did not occur in any of the randomly selected field sites.

The properties of the 1510 host trees are categorised in Table 2.2 as follows: species of tree; height of tree (<2, 2-6 or >6m); and whether or not the growth of the tree had been manipulated (see methods). Each combination of properties has also been assigned a category (Cat) number from 1 to 21 for ease of reference. For each of these 21 categories, Table 2.2 lists the number of trees examined (N), the number of trees with *P. dorsalis* larvae (n), and the percentage (%) of trees with *P. dorsalis* larvae. The properties of each of the 1510 individual trees are not given. It should be noted in Table 2.2 that 4 unrelated specis, *E. gomphocephala, sideroxylon, porsa* and *diversifolia,* have been pooled as *E."other"* because of the small number of each in the samples. Four host tree species are therefore listed (*E. camaldulensis, leucoxylon, torquata,* and *other*) each with 6 possible categories of properties (manipulated x 3 heights, plus natural x 3 heights). For *E. torquata,* only 3 categories are listed, as no naturally growing *E. torquata* occurred in samples.

It is clear from Table 2.2 that the incidence of *P. dorsalis* is far greater on manipulated than on naturally occuring trees; manipulated trees up to 6m high account for 78% of all the trees on which *P. dorsalis* was recorded. On the natural trees, only in one category (Cat. 16; *E. other*, <2m) were larvae recorded reguarly (45% of trees infested), and the number of natural trees with larvae in the other categories is too small to allow further analysis.

Thus, further analysis was undertaken for the manipulated trees only. For such trees, the first hypothesis tested was that the presence of *P. dorsalis,* the species of tree and the height of tree are mutually independent. A three dimentional contingency table analysis was highly significant (Chi-square = 119.48; d.f. = 17; P <0.001; Appendix 2.3), and indicates that the 3 variables are not mutually independent. A further three dimentional contingency table analysis was undertaken to test for the partial independence of the presence of *P. dorsalis* of the species and height of tree (Appendix 2.4). A highly

Table 2.2

The number of trees examined (N), the number of trees with P. dorsalis (n), and the percentage (%) of the number of trees with P. dorsalis in each of 21 categories (Cat.) of trees. The categories have been numbered for ease of reference within the text.

Manipulated													
Tree height	<2m				2-6m				>6m				
Tree species	Cat.	N	n	%	Cat.	N	n	%	Cat.	N	n	%	Mean % for sp.
E. camaldulensis	1	41	20	48.8	2	135	37	27.4	3	130	6	4.6	26.9
E. leucoxylon	7	39	6	15.4	8	37	8	21.6	9	12	0	0	12.3
E. other	13	9	8	88.9	14	12	4	33.3	15	8	0	0	40.7
E. torquata	19	21	5	23.8	20	38	9	23.7	21	14	0	0	15.8
Mean % for height				44.2				26.5				1.2	

Natural													
Tree height	<2m				2-6m				>6m				
Tree species	Cat.	N	n	%	Cat.	N	n	%	Cat.	N	n	%	Mean % for sp.
E. camaldulensis	4	51	0	0	5	133	8	6	6	623	3	0.5	2.17
E. leucoxylon	10	48	0	0	11	42	0	0	12	70	0	0	0
E. other	16	20	9	45	17	25	1	4	18	2	0	0	16.3
E. torquata													
Mean % for height				15				3.3				0.17	

significant result indicates that the presence of *P. dorsalis* is dependent both on the species and on the height of tree (Chi-square = 145.16; d.f. = 11; P <0.001). For *E. camaldulensis* only (the species of which by far the most trees were examined), the presence of *P. dorsalis* is dependent on the height of tree (Contingency table analysis, Chi-square = 44.058, df=2, P <0.001; Appendix 2.5). Short trees had by far the highest incidence of *P. dorsalis* (49% infestation); the 2-6m trees had fewer larvae (27% infestation), and only 4.6% of *E. camaldulensis* trees >6m had any larvae at all (Table 2.2; Appendix 2.5). For the remaining species of *Eucalyptus*, there were too few trees in the samples to allow a similar statistical analysis; the figures for these species (Table 2.2) neverless appear to follow the same trend.

For each height class of tree, the further hypothesis was tested that the presence of *P. dorsalis* was independent of the species of tree. For trees <2m, the presence of *P. dorsalis* was dependent on the species of tree (Contingency table analysis, Chi-square = 22.52, df=3, P <0.001; Appendix 2.6a). The highest incidence of *P. dorsalis* was recorded on *E. other* (89% of trees infested), and the incidence on this group of species was significantly higher than the incidence on any other species (log-likelyhood ratios, Appendix 2.6). *E. camaldulensis*, in which 49% of trees were infested, had a significantly higher incidence of *P. dorsalis* than did *E. leucoxylon* (15%); the remaining differences between species were not significant (log-likelyhood ratios, Appendix 2.6a). For 2-6m trees, the presence of *P. dorsalis* was not dependent on the species of tree (Appendix 2.6b). Finally, for trees > 6m, *P. dorsalis* larvae were recorded only on *E. camaldulensis* (4.6% of trees infested), but very few trees of other species were encountered in this height category, and very few were consequently examined (Appendix 2.6c).

2.4 Discussion

There was clearly a predominance of *P. dorsalis* in areas centered on the 700 mm isohyet, which is somewhat wetter than the 550 mm (22 inches) reported for the related *P. affinis* in New South Wales (Carne, 1969). Carne (1965) suggests that a critical factor leading to such a distribution of sawflies is the inability of mature larvae to penetrate soil which has dried to a hard crust; in areas where low rainfall and soil type combine to result in the formation of such crusts, larvae cannot aestivate in the soil, and they perish. Conversely, cocooned larvae may drown when prolonged water logging of the soil occurs, as has been demonstrated for *Helicoverpa punctigera* (Wallengren) (Murray and Zalucki, 1990). In wet soils, larvae may also suffer significant mortality from fungal infection, as is demonstrated in Chapter 6. However, larvae of *P. dorsalis* are likely to be present in some areas where survival of the cocooned stages is not possible, because adults can disperse from more favourable areas nearby.

The proximity of favourable areas may therefore explain the presence of *P. dorsalis* in some marginal areas. *P. dorsalis* was recorded, for example, at Murray Bridge, Wellington and Langhorne Creek, all of which have an annual rainfall of less than 400 mm. However, there were no records of *P. dorsalis* at Kadina, Moonta or Mallala, which have similar rainfalls to the towns above, but which are much further from a 700 mm isohyet (see Fig. 2.1).

Of those eucalypts native to the regions surveyed, *E. camaldulensis* was the host species on which *P. dorsalis* larvae were most frequently recorded. Interestingly, *P. dorsalis* had an even higher incidence on the pooled group of four rarer species recorded as 'other'. Of the latter, *E. gomphocephala, E. sideroxylon* and *E. diversifolia* are not found naturally in the areas surveyed (i.e. they are 'non-native'), and *P. dorsalis* is therefore found most frequently upon non-native eucalypts. It is possible that these species of *Eucalyptus* are less resistant to *P. dorsalis* than are species upon which *P. dorsalis* may have evolved. Alternatively, the non-native trees may have been 'stressed' in some way, as a result of growing in a physical and climatic environment to which they are not adapted; *P. dorsalis* may then have occurred on them more frequently as a result of increased oviposition or survival

on 'stressed' trees. The preference of herbiroves for stressed plants has been reported extensively (for example; Heinrichs, 1988; Bernays, 1989), and may result from changes in foliar levels of nitrogen or other metabolites (White, 1969, 1974; Heinrichs, 1988; Weinstein, 1990).

If the incidence of *P. dorsalis* is, in fact, higher on 'stressed' trees, this may also in part explain the prevalence of the insect on 'manipulated' trees; metropolitan trees which were subject to pruning, overwatering, shading and air pollution were all categorised as 'manipulated' in the survey. In addition to the first three of these stresses, which are well recognised (Heinrichs, 1988; Landsberg, 1990; Wagner and Frantz, 1990), air pollution has recently been shown to significantly affect insect-plant interactions by roadsides (Spencer *et al.*, 1988; Riemer and Whittaker, 1989). Spencer *et al.* (1988) demonstrated that nitrogen content and aphid numbers were higher on roadside plants than on more distant controls, and roadside eucalypts and *P. dorsalis* larvae may be similarly affected.

On the other hand, Moore *et al.* (1988) point out the importance of environmental influences on oviposition behaviour, questioning the validity of the common assumption that distribution is a reflection of variation in food quality. The apparency of trees to the ovipositing female may, for example, be a more significant determinant of egg distribution than is the quality of the host tree as a food source for the larvae. The small, manipulated trees in this survey had often been planted only recently at exposed sites such as in parks and gardens or by the road side, and these young trees may have had a higher incidence of *P. dorsalis* larvae because the young trees were not hidden by a natural canopy of older trees.

A combination of factors probably offers the most plausible explanation of the higher incidence of *P. dorsalis* larvae on small, manipulated, non-native trees. Obviously, late instar *P. dorsalis* larvae will be found in a eucalypt only if the female found the tree to oviposit onto, and only if the resultant eggs and larvae survived thereupon. The 'greenness' of young trees may be more apparent to adult females, or adult females may penetrate the young leaves more easily with their ovipositors. At the same time, small young eucalypts may have foliage which is more susceptible and acceptable

to sawfly larvae. Young foliage in eucalypts is known to be more susceptible to damage by other insects (eg. New, 1981; Landsberg, 1988), presumably because larval mortality is lower on young foliage than on more mature foliage.

Regardless of its cause, the high incidence of *P. dorsalis* on small, manipulated non-native eucalypts demonstrated in this study has obvious implications for the management of eucalypt plantations. The results indicate that young, even-aged monocultures of species growing outside of their natural habitat range will be extremely vulnerable to outbreaks of *P. dorsalis,* and there is evidence that such plantations are also vulnerable to attack by other eucalypt-feeding insects (Neumann, 1989). Vulnerability may be at least partially overcome by replanting *Eucalyptus* species within their natural range, possibly in the understory of existing but depleted woodlands.

CHAPTER 3

THE DEVELOPMENT OF *TAENIOGONALOS VENATORIA*.

CHAPTER 3

The Development of *Taeniogonalos venatoria*

3.1 Introduction

Developmental data for trigonalyids are scarce, and are available for only a few species. In order to understand the lifecycle and host relationships of *T. venatoria*, it was therefore necessary to document the development of this species in detail.

Developmental work that has been previously undertaken on other species is first summarised, and a number of generalisations about the biology of the family are made. The results of this study on the development of *T. venatoria* are then presented, and are compared and contrasted to details available for other species. The factors leading to egg eclosion are investigated experimentally for the first time for any trigonalyid. Adult emergence phenology and the effects of feeding on longevity and fecundity are also described for the first time for any trigonalyid.

3.2 Review of Trigonalyid Development

(Published in part, Weinstein and Austin 1991; see Appendix 1.)

3.2.1 Oviposition and Egg morphology

Oviposition on foliage was first assumed for trigonalyids by Wheeler (1928) on the basis of the enormous number of eggs in their ovaries. The prediction was confirmed by Teranishi (1929) who published observations of *Poecilogonalos maga* Teranashi ovipositing on bamboo leaves. This unique oviposition strategy is now recorded for a number of species from different genera, and is thus thought to be a family level character. In brief, females walk along the upper surface of a leaf, rotate so their long axis is perpendicular to the leaf margin, and curl their gaster around to the underside of the margin. In some species the tip of the gaster is used to punch a hole in the epidermis, with an egg being left in the mesophyll layer, at the base of a slit-like incision less than one millimetre from the margin (Vecht, 1933; Rodd, 1951; Townes, 1956). Other species simply deposit eggs on the leaf epidermis, to which they adhere only lightly (Clausen, 1929; Townes 1956). The former method is linked to the presence of a process on the second or third gastral sternite, which is placed on the upper surface of the margin, directly opposite the insertion site. This process is thought to stabilise the leaf during oviposition, providing a pressure point whilst a hole is punched in the epidermis from below (Townes, 1956). Rodd (1951) further suggests that the sternal process and tip of the gaster act as a pair of calipers, gauging the depth of oviposition into the mesophyll. By either method, the whole oviposition process is completed quickly, and can be repeated almost immediately. Published estimates of oviposition rates are one oviposition every four seconds (*Orthogonalos pulchella* (Cresson); Townes, 1956) and 27 ovipositions per minute (*Poecilogonalos thwaitesi* (Westwood); Clausen, 1929). Eggs are always placed close to the leaf margin, consistent with the behaviour of most folivorous insect larvae of eating the edge first.

The eggs are microtypic and range from 0.1 to 0.15 mm long and 0.05 to 0.07 mm wide. They are typically ovoid, arched dorsally, and demonstrate a patterning of longitudinal ridges which ranges from 5-7 uniform lines to an irregular interconnecting system (Clausen, 1931; Rodd, 1951). Those species that have been recorded as ovipositing on the epidermis typically have eggs which are flat ventrally (Clausen, 1931), whereas eggs oviposited in the mesophyll are convex on both sides (Vecht, 1933). The eggs of *Taeniogonalos maculata* (Smith) appear discrepant in this regard, being flat ventrally and oviposited into the mesophyll (Rodd, 1951). However, the interpretation of "flat ventrally" is open to question for the latter species, as the state of hydration appears to influence the shape of the egg considerably. Rodd (1951) describes how the ventral chorion balloons out upon immersion in water, and it is possible that the two different shapes result from increased water availability to eggs placed in the mesophyll. In contrast, the dorsal chorion is exceedingly tough, and it has been suggested that the interplay of these two surfaces leads to a reduced mortality when the eggs are subjected to the mandibular action of their primary (phytophagous) host larva (Rodd, 1951). Teranishi (1929) is the only author to indicate that trigonalyid eggs have a micropyle, stating that the "micropylar protuberance is prominent" in the eggs of *P. maga* (a species ovipositing on the leaf surface).

Wheeler (1928) predicted that trigonalyid eggs, apart from being oviposited on foliage, would also eclose there and give rise to a planidial type larva. Clausen (1929) observed eggs upon foliage for several months, periodically examining leaves for the presence of viable larvae, and yet found none. He concluded that eclosion was dependent upon the consumption of the eggs by the host, in a manner that was already known for some tachinid flies, and he was the first to demonstrate this experimentally. He showed that treatment with a weak solution of KOH led to the emergence of larvae, but only from eggs in which the chorion was cracked (Clausen, 1931). Slight pressure on the eggs, exerted with a coverslip, resulted in the development of longitudinal splits between the surface ridges, suggesting their role may be to enable chorionic cracking, without shattering the egg and crushing the embryo within. Clausen concluded that a combination of physical and chemical stimuli from the host

mandibles and digestive secretions lead to the eclosion of trigonalyid larvae. He confirmed his observations *in vivo* by feeding the eggs of *P. maga* and *Satogonalos debilis* (Teranishi) to papilionid larvae on foliage. Dissections after one hour revealed free first instar larvae in the anterior portions of the gut; at 4 hours a few of these were found in the body cavity. Eggs with intact chorions were also recovered from the resultant frass, and contained viable larvae upon dissection (Clausen, 1931).

3.2.2 Larval Development

First Instar

The first instar, having eclosed in the gut of the primary host, is assumed to penetrate the gut lining and enter the haemocoel. Clausen (1931) recovered larvae from the haemolymph of papilionid and sawfly larvae some hours after these had consumed trigonalyid eggs. If a another parasitoid (i.e. ichneumonid or tachinid larva) is also present within the phytophagous host, the trigonalyid larva is presumably either ingested as the other parasitoid consumes the phytophagous host's tissue, or the trigonalyid larva may penetrate directly through the cuticle of the other parasitoid (secondary host) to gain access to the haemolymph. Alternatively, the phytophagous (primary) host may be used to provision the cells of developing vespid or eumenid larvae (see Chapter 4). The trigonalyid larvae are then assumed to penetrate the vespid or eumenid larva's gut wall after being ingested with the tissues of the phytophagous host, or after being fed to the larva with the regurgitate of an adult wasp. Regardless of which is the final host, further development of the trigonalyid larva is delayed until the final host either pupates or approaches pupation, having at least completed the formation of its cocoon or having been sealed in its cell (Clausen, 1940; Riek, 1962b; Yamane, 1973). In the case of *Taeniogonalos* spp., the phytophagous (primary) host also completes cocoon formation, such that the trigonalyid emerges from the cocoon of the other parasitoid (ichneumonid or tachinid) within the cocoon of an anthelid moth (Riek 1962b) or pergid sawfly (Weinstein and Austin, 1991).

The first instar has only been described for *P. maga* and *S. debilis* (Clausen, 1931). It is about 0.12 mm long and has the head largely

retracted into the thorax. It possesses slender mandibles and, on the first thoracic segment, has a transverse ventral row of exceedingly heavy hooks directed posteriorly. The second and third thoracic segments have rows of heavy spines both dorsally and ventrally, and the abdominal segments have rows of minute setae which completely encircle the last two or three segments. These structures presumably facilitate the larva's passage through the gut wall of its primary host, or through either the gut wall or cuticle of its secondary host.

Second Instar

The second instar is about one millimetre long and possesses a large globular unchitinised head (*P. thwaitesi,* Clausen, 1929). The mandibles are widely spaced and are either simple (Clausen, 1929) or possess rows of minute teeth medially (*Bareogonalos jezoensis* (Uchida); Yamane, 1973). The body segments have no hooks or setae (Clausen, 1940).

Third Instar

The third instar is 3–4 mm long, and possesses a large heavily chitinised head capsule with huge mandibles (Clausen, 1929; Yamane, 1973). The enlarged mandibles are used to lacerate and kill superparasitoids, but a larva may also perish as a result of being internally parasitised by conspecifics (Clausen, 1929). The latter is not the normal method by which solitary parasitoids eliminate superparasitoids (Askew, 1971), and shows an extreme lack of host specificity (see below).

Fourth Instar

The fourth instar is more than 5 mm long, has a thick abdomen, lightly chitinised head capsule, and smaller, simpler mandibles than the third instar (Clausen, 1929; Yamane, 1973). It is during this stage that trigonalyid larvae become ectoparasitic. The pre-moulting third instar or early fourth instar assumes a position just beneath the dermis of the host pupa or prepupa, either within the eye or in the thoracic region. The fourth instar emerges from this point, and feeds initially at the emergence hole (Clausen, 1929; Cooper, 1954; Gauss, 1962; Yamane, 1973). Death of the host follows, and new feeding incisions may later be cut elsewhere in the host remains.

Fifth Instar

The fifth instar is over 10 mm in length, has a robust body and distinctive tridentate mandibles (Clausen, 1929; Cooper, 1954; Yamane, 1973; Yamane and Kojima, 1982; Yamane and Terayama 1983). Authors differ in their assessment of the importance of feeding in this stage. Clausen (1929) states that the fifth instar of *P. thwaitesi* feeds very little and, despite the heavy mandibles, is limited to sucking up fluid from what remains of the host. However, Cooper (1954) says that the majority of feeding is carried out by the fifth instar of *Lycogaster pullata* Shuckard. In this species the larva almost tripled in size in three days, became so bloated as to lose all signs of body segmentation, and "crushed, chewed and plied the soft pulpy tissues of the host". The size of the host may influence how much feeding occurs during the fifth instar, but in both cases approximately half of the host tissues were left untouched. This is surprising in view of the apparently fierce competition for resources that can occur during the third instar.

Pupa

At the completion of feeding, the fifth instar may construct an irregular cocoon, a silken partition, or no cocoon at all. *P. thwaitesi* spins an irregular brown parchment-like cocoon within that of its ichneumonid host, thus partitioning off the degenerating remains of the latter (Clausen, 1929). *B. jezoensis* constructs a silken cross-wall in the cell of its vespid host, allowing workers to clean the remaining part of the cell (Vecht, 1933; Yamane, 1973). *T. maculata, L. pullata* and *Pseudonomadina biceps* Yamane & Kojima construct no cocoon at all, remaining free within the host cocoon (Raff, 1934; Cooper, 1954; Yamane and Kojima, 1982). The fifth instar then becomes quiescent, and within one or two days passes a viscid brown-black meconial sac. The sac contains a clear supernatant fluid over finely-textured solids, and remains connected to the tip of the abdomen by an integumentary strand (Clausen, 1929; Cooper, 1954). Cooper (1954) suggests that this sac is formed from the impervious membrane lining the larval hindgut rather than from the peritrophic membrane. Pupal eclosion occurs one day or so after the meconium is passed, and the fifth instar exuvium remains partially enveloping the legs and abdomen of the pupa. Body coloration is complete within 8

days, and loosening and wrinkling of the pupal envelope precedes the final eclosion a further day later (Cooper, 1954).

3.2.3 Emergence Phenology

Adult trigonalyids have dentate mandibles, and make use of these to cut neat round emergence holes in either the side of the host cocoon or puparium (Ichneumonidae, Tachinidae, Lepidoptera) or through the top cover of the cocoon or cell (Symphyta, Vespidae). As trigonalyid larvae complete development only on host pupae or prepupae, their emergence is necessarily synchronised with that of the host (Clausen, 1940; Riek, 1962b; Yamane, 1973). Actively feeding primary host larvae are therefore available at or shortly after the time at which trigonalyids oviposit onto leaves. The mechanism of this synchronisation has not been studied, but it is more than likely linked to hormonal changes in the host (Beckage, 1985).

In temperate regions, emergence (as determined by adult collection data) tends to occur in spring or summer, with both primary host and trigonalyid being univoltine. One of the most detailed studies remains that of Clausen (1929) for *P. thwaitesi,* undertaken in Assam, India. The ichneumonid secondary host (*Henicospilus rufus* Tosquinet) emerges from its cocoon in the soil in March, with the trigonalyid following 1 to 12 days later. The ichneumonid starts parasitising lepidopteran larvae, slightly ahead of the trigonalyid ovipositing on foliage. In early winter (December) ichneumonid cocoons contain mature ichneumonid larvae and first instar trigonalyids, and in late winter (February) they contained developing trigonalyid larvae. The Australian *T. maculata* is interesting in that emergence occurs in March to May (autumn), the host being the winter-feeding sawfly *Perga* sp. (Raff, 1934; Rayment, 1952). *T. venatoria*, whose emergence phenology is presented in detail later in this chapter (see 3.4.3), was noted by Carne (1969) to have a similar emergence pattern. He also noted that *T. venatoria* could remain in diapause within its diapausing pergid host for at least two seasons, and still emerge in autumn (Carne, 1969; Danks, 1987; see also Chapter 6).

The only apparent exceptions to this temperate emergence pattern occur in species of *Bareogonalos* parasitizing vespids. Yamane (1973) records three male *B. jezoensis* taken in early June in Hokkaido, Japan.

Cocoons of its vespid host are generally not yet available at this time of the year, and he suggests that these individuals overwinter as adults. Carmean *et al.* (1981) also suggest that female *Bareogonalos canadensis* (Harrington) overwinter as adults, since they emerge after their vespid hosts have completed the rearing of the season's brood (August to September - Carmean *et al.*, 1981; September to October - Stage and Slobodchikoff, 1962). If adults do overwinter, it is possible that they remain in the nests of their host, as the wasps apparently do not attack them (Carmean *et al.*, 1981; Yamane and Kojima, 1982). However, in view of the short adult life span of other trigonalyids, it would appear more likely that late emergers proceed to oviposit, leaving the hardier and longer lived egg stage to overwinter in persistent foliage. Tsuneki (1975) and Nozaka (1976) (quoted in Matsuura and Yamane 1984) do in fact report *B. jezoensis* to be actively ovipositing in late autumn, in Honshu, Japan.

In tropical regions the phenology of trigonalyids is apparently more variable. To a large extent, the length of the life cycle may depend on how soon after oviposition a multivoltine host consumes trigonalyid eggs (Clausen, 1940). There are insufficient records to make any significant comparisons between host and parasitoid emergence dates, and tropical trigonalyids remain generally poorly studied.

3.2.4 Adults : Longevity, feeding, behaviour, sex ratio and fecundity

Adult trigonalyids are most commonly found in moist shady woodland habitats and in montane forests (Clausen, 1929; Vecht, 1934; Popov, 1945; Townes, 1956). They are usually seen crawling on foliage or taking short flights about one metre above the ground, amounting to no more than 'jumping' between bushes. They are diurnal and solitary, and move with quick jerky movements. When walking, feeding or resting, adults hold their wings posterolaterally, at right angles to each other; the abdomen is sometimes also raised at 45^{o} to the horizontal (Clausen, 1929; Haeseler, 1976). During prolonged rest, such as at night, they may fold their wings one above the other, held horizontally over the abdomen. The antennae vibrate rapidly except when at rest (Townes, 1956). Adults are short-lived with the longest recorded survival being that of a female *P. thwaitesi* which lived for

14 days (Clausen, 1931). They are commonly believed not to feed (e.g. Oehlke, 1983a), but this is clearly not true for some species at least. Clausen (1929) describes the posture of *P. thwaitesi* while feeding, Townes (1956) records *Poecilogonalos costalis* (Cresson) as feeding on honey dew, while Bugnion (1910) describes the digestive tract of *Trigonalis hahni* Spinola as appearing functional.

The size of adult trigonalyids is known to vary with the size of the host it has developed upon; the size of *Bareogonalos huisuni* Yamane and Yamane for example is dependent upon the cast of vespid cell from which it emerges (Yamane and Yamane, 1975). Specific characters are also variable, and adult *B. jezoensis* reared from *Vespula* look specifically different to those reared from *Vespa* (Matsuura and Yamane, 1984).

Although mating has not been described, it is assumed that most species reproduce sexually on the basis that (1) the anatomy of the male reproductive organs indicates that they are functional (Bugnion, 1910), (2) males are sometimes mutilated when paired individuals are caged (presumably after males attempt mating) (Clausen, 1929), and (3) a significant percentage of males occurs in most populations. Female to male sex ratios vary from approximately 1:1 in *Orthogonalos pulchella* (Cresson) (Townes, 1956), *B. huisuni* (Yamane & Yamane, 1975), and *B. canadensis* (Carmean, pers. comm.), to more than 1000:1 for *T. venatoria* (Carne, 1969). The latter species is probably the exception rather than the rule, and is assumed to be facultatively parthenogenetic.

With the precarious method by which hosts are parasitised, one would expect trigonalyids to be very fecund, and there is good evidence to indicate that this is indeed the case. Bugnion (1910) counted 3-400 ovarioles in each ovary of *T. hahnii,* with 5 mature eggs, and others developing in each ovariole, giving at least 3000 eggs. Clausen (1929) counted 8,218 eggs (with more developing) in *P. thwaitesi,* while actual numbers of ovipositions reported are even more impressive. The latter include 3,559 in four days (*P. maga*; Teranishi 1929), 5,782 in 6 days (*Poecilogonalos henicospili* Rohwer; Clausen 1929) and 10,641 in 14 days by *P. thwaitesi*; the latter species having also been recorded as laying 4,376 eggs in one day (Clausen, 1931). It is clear from these figures that eggs develop during the lifetime of an adult

female, and that a total fecundity of 10,000 or more eggs is not an unreasonable estimate for many species (Clausen, 1931; Oehlke, 1983a).

Most of the species on which the above generalisations are based are non-Australian parasitoids of vespids, and it is therefore desirable to test the hypothesis that these generalisations are equally applicable to the Australian *T. venatoria*, which does not parasitise vespids. A study of the development of *T. venatoria* therefore follows.

3.3 Materials and methods

3.3.1 Egg morphplogy

Eggs were obtained from the leaves of healthy potted 2m *Eucalyptus camaldulensis* trees on which caged *T. venatoria* had oviposited during April 1989.

Eggs for SEM examination were dissected from the leaves with a fine needle. They were placed directly onto double-sided sticky tape on EM stubs, and were examined on a Cambridge Stereoscan 250 MKIII at magnifications of x500 and x1000.

Eclosion

In this experiment, the physiological cues that may be encountered by ingested eggs were simulated *in vitro* in order to determine which of these cues may be the most successful stimulant of eclosion. Eggs for eclosion trials were dissected as above, and were placed into the open ends of 1 µl. microcaps containing saline (0.9% NaCl). The opposite ends of the microcaps were partially blocked with micropins. Using a 10 µl. glass syringe, fluid could thus be passed over the eggs without these being flushed out of the microcap (Fig. 3.1.). Thirty eggs were inserted into each of 28 microcaps over a period of 10 weeks. Each microcap was irrigated with a different combination of the following fluids:

(1) The supernatant of dissected, homogenised and centrifugend fore-guts and salivary glands from 4th instar *P. dorsalis* larvae,

(2) Saline buffered to pH 7.5 using Tris buffer (Sigma T-1378),

(3) 2% trypsin in saline (Sigma T-0134),

(4) Saline brought to pH 6.0 using $H_2 SO_4$,

(5) 2% cathepsin in saline (Sigma C-6286),

(6) Gaseous CO_2 in a sealed plastic container into which the microcaps were placed.

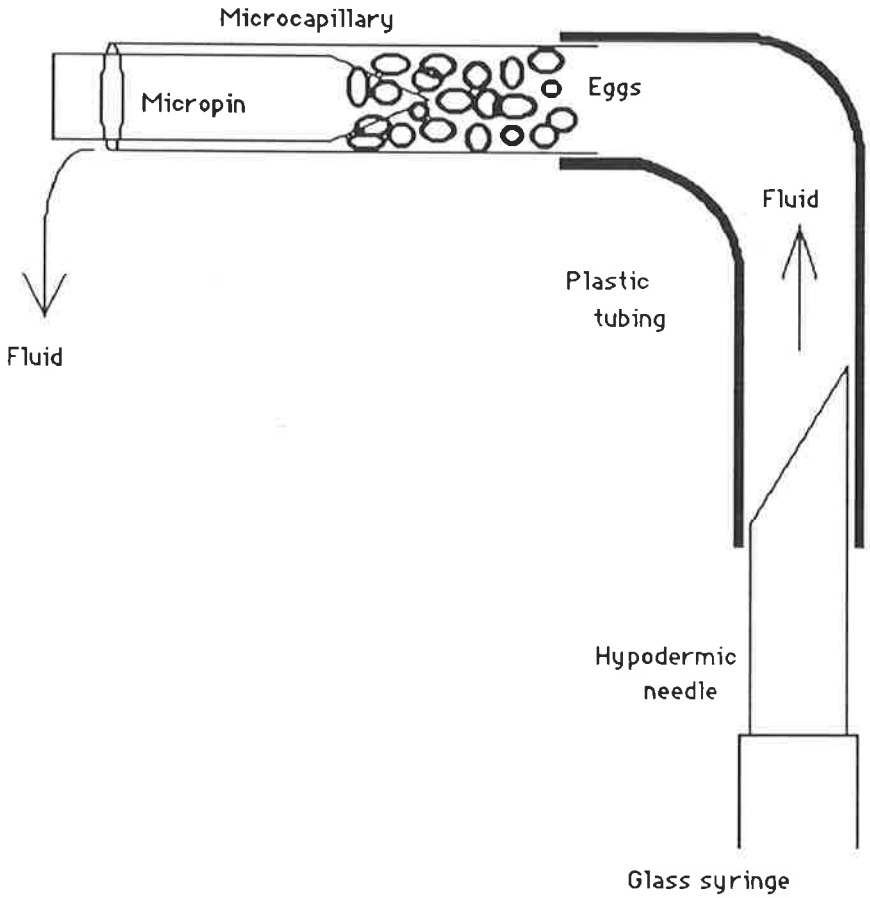


Figure 3.1 Setup for the irrigation of eggs with various fluids.

Fluid (1) had a pH of 6.0±0.5 (N=3, Neutralit pH strips, Merk), the same as that of sawfly gut contents (6.0±0.5, N=3, Neutralit pH strips, Merk).

Ten different combinations of these fluids were used, as set out in Table 3.1, constituting the 10 treatments to which the eggs were subjected. The treatments were selected to stimulate potential host gut conditions in *P. dorsalis*, in a host with alkaline gut contents, and in a host with acidic gut contents, both under aerobic and anaerobic conditions.

Two microcaps (replicates) were allocated to each of the ten different treatments. To increase the number of replicates, selected treatments were repeated on microcaps containing fresh eggs after a varying number of weeks. The treatments and replicates are given in chronological order in Appendix 3.1. In order to control for the effects of needle manipulation and insertion into capillaries, two controls (A and B) were included. In these controls, the eggs were treated with the same fluids used in treatments 4 and 9, but without needle manipulation (Table 3.2). The eggs were left *in situ* in the leaf tissue and were exposed by gently enlarging the oviposition slits above them. Approximately 30 eggs in each replicate were then covered with drops of the appropriate fluids. The leaf sections were kept in sealed petri-dishes, to avoid evaporation of the treatment fluids.

All eggs were examined daily with a dissecting microscope, and the total number of eclosions in each microcap was recorded seven days after the start of treatment. The hypothesis that no treatment resulted in more eclosions than did other treatments was tested by log-likelyhood ratio (G) test.

3.3.2 Larval Development

Eggs were eclosed as described in the previous section. Emergent first instar larvae were mounted on slides in Hoyer's medium, and were observed under a compound microscope at a magnification of x400. Other larval stages were obtained by dissecting late instar host larvae, prepupae and pupae which had been reared in captivity on foliage containing the eggs of *T. venatoria*. Late instar larvae are

ectoparasitic (see 3.2.2), and these stages were observed feeding upon host pupae which had been placed in transparent plastic vials, as described in section 4.2 (Host relationships).

The numbers of instars was determined on the basis of morphological differences between the mandibles (Clausen, 1929, 1931; Cooper, 1954; Yamane, 1973; Yamane and Kojima, 1982). Development times were recorded where possible, and notes were made on pupation and emergence. All observations were for larvae of *T. venatoria* acting as primary parasitoids, as defined in section 4.3.

3.3.3 Emergence Phenology

The presence of the different developmental stages of *P. dorsalis* and *T. venatoria* were recorded in the Adelaide region from 1986 to 1990. Consistent recording times and places were not used, and data were recorded cumulatively as they became available through surveys (Section 2.2), laboratory rearings, dissections, field collections, and casual observations. The developmental data for *T. venatoria* larvae are from laboratory rearings of field collected mateial, as well as from sawfly larvae exposed to trigonalyid eggs on foliage in field cages. To determine the number of years that *T. venatoria* was capable of diapausing for, emergences were recorded in each successive year from cocoon masses that formed in 1987.

3.3.4 Adults: Sex ratio, fecundity, feeding, and longevity

Several thousand adult *T. venatoria* were reared during 1987-1990, of which only 8 were males (see Chapter 1). Matings were attempted in plastic vials, and on potted *E. camaldulensis* in field cages; both naïve females and females with ovipostion experience were used. The number of eggs in the ovaries were counted upon dissection of 8 newly emerged females, and 6 females with 3 days of known oviposition experience on *E. camaldulensis*. The latter females were fed honey *ad libidum*, and their individual behaviour was video recorded in a $1m^3$ cage made of mosquito-netting, which contained the foliage of *E. camaldulensis*. Upon replaying these recordings and examining the foliage, it was established that each female had laid a minimum of 600 eggs per day. The ovaries were macerated, and a total egg count

was undertaken for each female. Although tedious, this procedure was more reliable than subsampling ovarioles. The latter are very numerous and extemely fragile, and therefore virtually impossible to dissect consistently. Neither was it possible to subsample microscopic fields; the remnants of ovariolar strands caused irregular clumping of the eggs, and they could not be distributed uniformly.

Both male and female adults were presented with smears of water, honey, and pollen on the cage netting, and their responses were noted. Feeding behaviour was also observed in naturally occurring females in the field. During April 1988, the influence of honey on the longevity of females was determined in an experiment in which the individuals in each of 16 pairs of females were kept in adjacent $1m^3$ field cages (as above), with daily temperatures generally between 20 and 35°C. One female within each pair was supplied with honey and water, and the other, for comparison, was supplied with water only.

During hot weather (≥30°C) , individuals perished within 24 h without water, so water-soaked cotton was available to all individuals at all times.

3.4 Results

3.4.1 Egg morphology

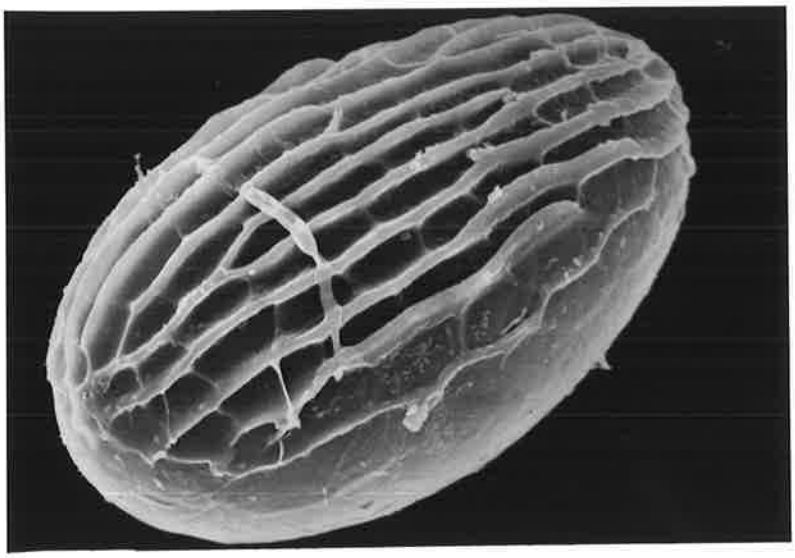
The eggs of *T. venatoria* (Figure 3.2.) are ovoid and measure 0.135 mm (SE=0.002, N=9) by 0.083mm (SE=0.003, N=11). Examination with the S.E.M. shows the dorsal surface to be covered with 10-11 major longitudinal ridges, which are irregular, and interconnect both by branching and by minor ridges running at right angles to them. The patterning occupies about one third of the egg surface. The ventral and lateral surfaces are smooth and round. There is no evidence of a pedicel.

Eclosion

The average percentage eclosion for each treatment is given in Table 3.1 and the number of eclosions in each replicate is listed chronologically in Appendix 3.1.

As treatments 1, 4 and 9 together account for 39 out of the 43 eclosions (90.7% of eclosions - see Table 3.1), the hypothesis that each of these treatments resulted in more eclosions than did the remaining treatments combined was tested. The mean number of eclosions resulting from eggs in treatment 1 (saline only) was significantly greater than the mean number resulting from eggs in treatments 2, 3, 5, 6, 7, 8 and 10 combined ($G=20.92$, $df=1$, $P<0.001$). Similarly, there were more eclosions from eggs in treatment 4 (acid saline and cathepsin) and in treatment 9 (acid saline only) than there were from eggs in the remaining treatments combined ($G=31.65$, $df=1$, $P<0.001$, and $G= 12.29$, $df=1$, $P<0.001$ respectively). For replicates of treatments of similarly aged eggs (treated on or before 15/7, see Appendix 3.1), there is no significant difference between the number of eclosions in treatments 1, 4 and 9 (1 vs 4, $G=0.65$, $df=1$, NS; 1 v 9, $G=0$, $df=1$, NS; 4 vs 9, $G=0.43$, $df=1$, NS). Cathepsin without acid was not effective in hatching eggs (Treatment 10 vs 4: $G=6.64$, $df=1$, $P<0.01$), which is perhaps not surprising when the reduced activity of the enzyme at neutral pH is considered. These results indicate that saline only, acid saline and cathepsin, and acid saline only were equally effective in triggering eclosion in this experiment.

(a)



(b)

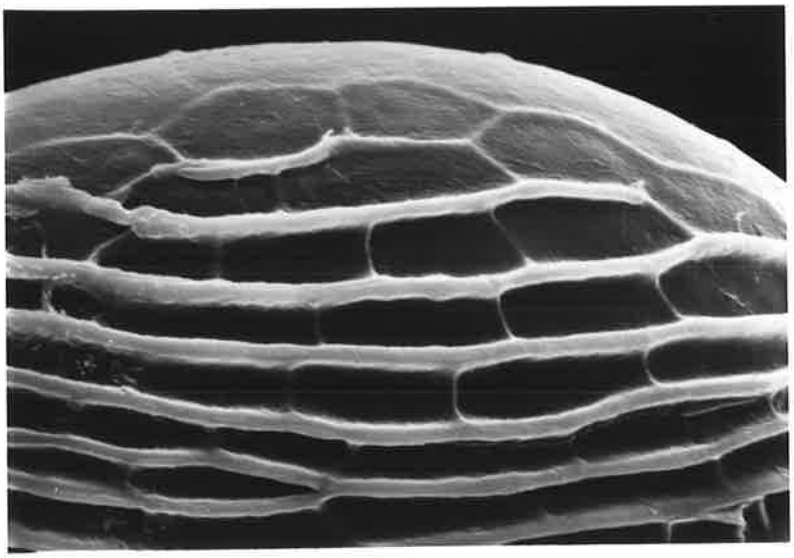


Figure 3.2.
Eggs of *T. venatoria* at x500 (a) and x1000(b), showing major longitudinal and minor latitudinal ridgeing. Some plant material remains adherent to the surface.

Table 3.1

Percentage eclosion in response to irrigation with various fluids : 10 different combinations of fluids (constituting the 10 treatments) are given, together with the number of eggs exposed (n), and the number (N) and percentage of eggs eclosed. The controls A and B were irrigated with the same solutions used in treatments 9 and 4 respectively, but the eggs had not been manipulated with a needle. Eggs in treatment 1 were not irrigated with any fluids other than the saline into which all eggs were originally placed.

Treat-ments	Homo-genate	pH 7.5 saline	Trypsin 2%	pH 6.0 saline	Chymo-tryp 2%	CO2 30 min	n eggs	N eclosed	% eclosed
1							120	12	10.00
2	+						60	1	1.67
3		+	+				60	0	0
4				+	+		120	16	13.30
5						+	60	0	0
6	+					+	60	0	0
7		+	+			+	60	0	0
8				+	+	+	60	2	3.33
9				+			180	11	6.11
10					+		60	1	1.67
Totals							840	43	5.12
Controls									
A				+			60	1	1.67
B				+	+		60	0	0
Totals							120	1	0.83

The lack of significant differences between the mean number of eclosions in treatments 1, 4 and 9 allows the eclosions from these treatments to be pooled in order to test the hypothesis that egg viability (as reflected in the number of eclosions) decreases with time. Taking 20/6/89 as day 1 of the experiment, the number of eclosions in each replicate of teatments 1, 4 and 9 are plotted against time in Figure 3.3. As might be expected, egg viability decreases with time ($r=-0.66$, $df=13$, $P<0.01$). The eclosion of an egg in treatment 9 during the first week of September (Appendix 3.1) demonstrates that some eggs remain viable in the field for at least 5 months.

By comparing eclosions from eggs in controls A and B to eclosions from similarly aged eggs in treatments 4 and 9 (replicates of 20/6 and 15/9 combined), it was possible to test the hypothesis that manipulating the eggs did not significantly affect the number of eclosions. A highly significant log likelyhood ratio ($G=17.09$, $df=1$, $P<0.001$) indicates that manipulation does affect the number of eclosions, with many more manipulated eggs eclosing. Treatment with CO_2 significantly decreased eclosion (treatments 1-4 combined vs 5-8 combined; $G=10.28$, $df=1$, $P<0.005$).

3.4.2 Larval Development

The number of instars, their size, duration and shape, and the size of their mandibles are summarised in Table 3.2. Brief biological notes on the development of each are presented below.

First instar larvae

0.2mm larvae similar to those described for *P. maga* and *S. debilis* (Clausen, 1931). Because of their small size and transparent nature, no first instar larave could be located in the haemocoel or intestinal wall of host larvae by gross dissection. Histological methods were not employed.

Second instar larvae

No second instar larvae were obtained by dissection. Attempts at rearing viable second instar larvae from first instar larvae kept in drops of host haemolymph failed. The existence of second instar larvae is here assumed on the basis of the larval mandible sizes, which jump dramatically from 5 to 371μm (Table 3.2). Its existence

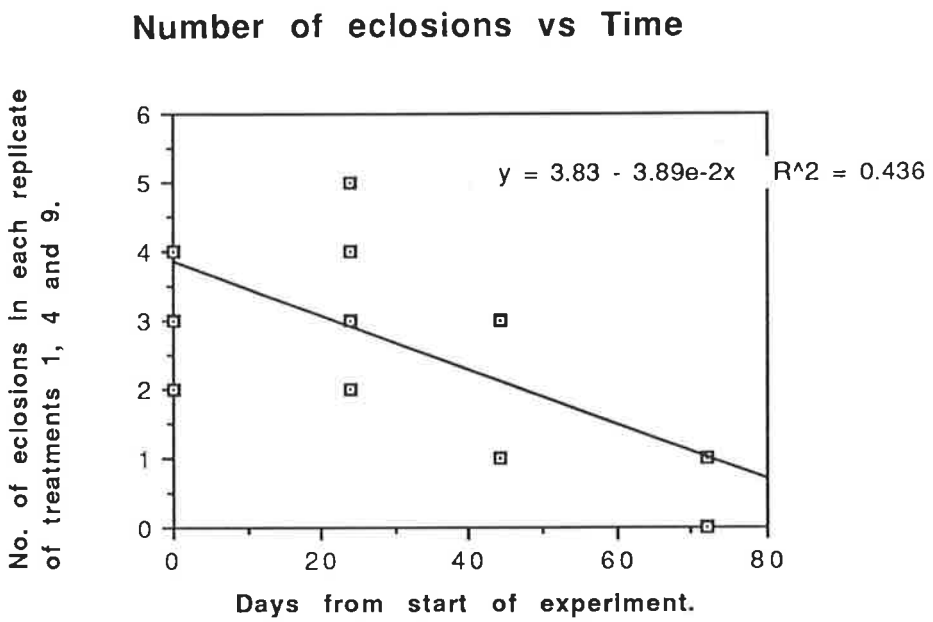


Figure 3.3.
The number of eclosions in each replicate of treatments 1, 4 and 9 (see Appendix 3.1) plotted against the number of days from the start of the experiment. There is a significant negative correlation between eclosion (egg viability) and number of days (time) ($r = -0.66$, $df=13$, $P<0.01$).

Table 3.2

The instar number, size, duration, and size and shape of the right (=left) mandible, *T. venatoria*.

Instar Number	L1	L2	L3	L4	L5	Pupa
Mandible Size (µm. ±S.D.; N=8)	5±0.5	- - -	371±9	226±8	350±22	- - -
Mandible Shape						
Larval Size (mm)*	0.2x0.05	- - -	1.5x0.7	3.0x1.0	10x4.0	10x3.0
Duration (days)	From 3 to 11 mths†	(?7)	(?7)	7±12h	7±12h	10±12h

*Approximate only as variation in the age of the instar and host size can lead to considerable variation in larval size.

†Several years in the case of hosts in prolonged diapause: refer 3.4.3 (emergence phenology).

is further supported by published descriptions of second instar larvae in other species of trigonalyids (Clausen, 1929; Yamane, 1973). These descriptions record the headcapsule as "unchitinised", which could explain the difficulty in locating larvae, or their exuviae, upon dissection.

Third instar larvae

1.5 mm larvae similar to those described for other species (Clausen, 1929; Yamane, 1973). Headcapulse measuring 568µ long by 695µ wide (S.E. 39µ, N=5), with mandibles 371µ long (S.E. 3.3, N=8). Large numbers of head capsules were found in individual host pupae, indicating that superparasitism is common. Clausen (1929) suggests that this heavily armed instar is responsible for the combatative elimination of superparasitoids. A few third instar larvae were found to be parasitising conspecifics (see Chapter 4: Host Relationships). Third instar larvae were found free in the haemolymph of host eonymphs, but were recovered predominantly from pupae (see Section 3.4.3: Emergence Phenology).

Fourth instar larvae

White 2-4 mm larvae similar to those described for other species (Clausen, 1929; Yamane, 1973). Mandibles simple, 0.2mm. All larvae emerged from the eye sockets or anterior thoracic regions of host pupae, remained at their emergence holes for 3-4 days, and fed as ectoparasites. From pupae in plastic vials, a second larva would sometimes emerge from the abdomen, ultimately giving rise to a second small adult. Such adults never emerged from naturally sealed host cocoons. The larva remains in the fourth instar for approximately 7 days.

Fifth instar larvae

5-10mm larvae, becoming increasingly cream to yellow in colour. Mandibles tridentate, similar to those described for other species (Clausen, 1929; Cooper, 1954; Yamane, 1973; Yamane and Kojima 1982). The fourth instar exuvium is extremely thin and transparent, and the moult is difficult to observe *in vivo*. The larva is no longer confined to near its emergence hole, and feeds from any part of the host's pupal remains. Unlike the fifth instar larva of *L. pullata*, and despite a rapid increase in the larva's size over 3-4 days, there was

no indication that it macerated or ingested host tissues other than fluids. The host tissues, although shrivelled, appear fresh until the completion of feeding, when blackening (and sometimes putrefaction) set it. After 5 days of feeding, the fifth instar becomes quiescent, and the pupal eyespots of a pharate pupa become visible under the cuticle on day 7.

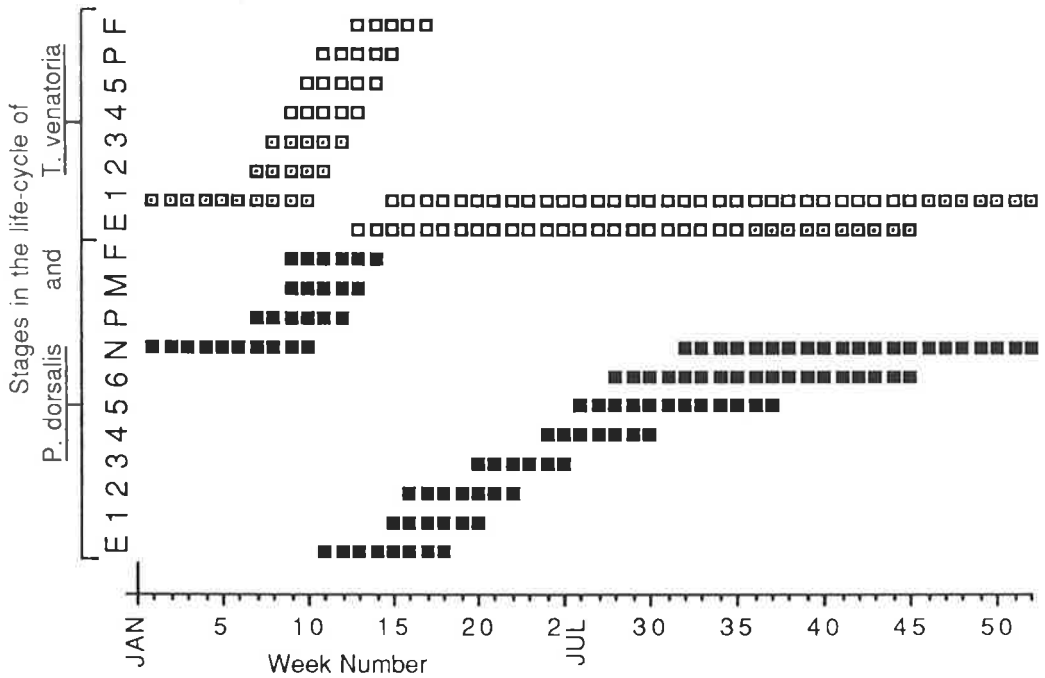
Pupa

A viscid white meconial sac appears at the anus on day 2, and a finely textured brown meconium is produced about 12 h later. The moult takes place a further 36 h later, and the meconial sack, still sealed in a water tight membrane, remains adherent (with the exuvium) to the tip of the pupal abdomen. No cocoon or partition is spun. Eyespots gained colouration after 2 days, followed by head and thoracic colouration (day 4) and finally abdominal colouration (day 6). On day 7 or 8 a fully formed and pigmented adult is clearly visible through the thin, translucent pupal membrane, and remains thus for a further 2 days before pupal eclosion. In dry plastic vials, adults were occasionally unable to withdraw their legs and antennae from the exuivum, and thus became trapped. *In vivo*, emerging adults chew a neat hole through the porous partitioning plate within the top of the cocoon, removing about one half of its area. They then force their way past the sixth instar larval host exuviae beyond this partition, and through the top of the cocoon and overlying soil to the surface. In dry soil, a neat emergence hole is apparent on the surface, the debris having been shunted backwards as the adult digs its way out.

3.4.3 Emergence Phenology

The presence of the different developmental stages of *P. dorsalis* and *T. venatoria* are summarised in Figure 3.4. For clarity, hosts and parasitoids undergoing prolonged diapause until the following emergence season(s) have not been included (see below).

Both *P. dorsalis* and *T. venatoria* are univoltine, and emerge from the soil and oviposit in autumn (March–April). *P. dorsalis* larvae feed in winter, and spend the summer months (September–February) diapausing in the soil. Both species complete development the following autumn, when adults again emerge from the soil. Individuals undergoing prolonged diapause emerge in synchrony with



Legend: ■ = *P. dorsalis*
□ = *T. venatoria;* ⊡ = assumed by deduction

E = egg
1-6 = L1 - L6
N = eonymph
P = pupa
M = adult male
F = adult female

Figure 3.4. The emergence phenology of *P. dorsalis* and *T. venatoria* based on field observations and laboratory rearings 1986-1990. The viability of trigonalyid eggs is based on eclosion experiments (see section 3.4.1). Individuals undergoing prolonged diapause have not been included for either species.

those of the succeeding generation(s) (not illustrated). Six female *T. venatoria* emerged after diapausing for **3** years (seasons). *P. dorsalis* larvae consumed the eggs in May 1987, and the adult female *T. venatoria* emerged between 26/3/90 and 11/4/90. Prolonged diapause is discussed in Chapter 6 and will not be dealt with further here.

3.4.4 Adults: Longevity, feeding behaviour, sex ratio and fecundity

Sex ratio

The sex ratio was less than 1:1000 for all *T. venatoria* reared in this study. All attempts at captive mating failed. In field cages, there was no apparent response between potential mates, and in confined vials they would mutilate each other upon making apparently random contact. The smaller males were rapidly dismembered, and attempts were discontinued in favour of preserving undamaged specimens. One damaged male was dissected, and had apparently functional reproductive organs, similar to those described for *Tr. hahnii* (Bugnion 1910). No attempt was made to look for viable sperm.

Fecundity

Egg counts made on newly emerged females, and on honey-fed females with 3 days of oviposition experience, are summarised in Table 3.3. Newly emerged females were found to contain about 6500 eggs. It is clear that more eggs develop during a female's lifetime: despite having oviposited at least 1800 eggs each, the post-oviposition egg counts did not differ significantly from those of newly emerged females ($t=0.18$, $df=12$, $p=0.86$). This finding is supported by the fact that developing eggs were located at the proximal ends of most ovarioles upon dissection. There was no evidence of egg reabsorption upon dissection of any female, including unresponsive females at the end of their lifespan (see below).

Feeding

Adults of both sexes readily took water and honey, particularly upon emergence. Drinking was observed frequently on hot days, and continued throught the lifetime of adults. Honey intake appeared to occur most readily during the first few days after emergence. No interest was shown in pollen (wet or dry) once water had been taken.

Table 3.3

The egg counts made on newly emerged females, and on honey-fed females with 3 days of oviposition experience.

Type of female	Number dissected	Mean egg number	Standard error*
Newly emerged	8	6467	582
3 days experience	6	6323	468

For comparison of groups, t=0.18, d.f.=12, p=0.86.

*The large S.E. probably relates mainly to the variable size of adult females.

Table 3.4

The longevity of 16 pairs of females held in identical field cages with access to either water and honey or water only.

Treatment	No. of individuals	Mean days survival	Standard error*
Honey fed	16	5.75	0.34
Water only	16	3.19	0.43

For comparison of groups, paired t=9.804, d.f.=31, p<0.001.

In the field, female *T. venatoria* were observed to feed on the honeydew of psyllids (*Glycaspis* spp.) on the foliage of *E. camaldulensis*.

Longevity

The longevity of 16 pairs of females is summarised in Table 3.4. The longest survival recorded was 8 days by two honey-fed females during relatively mild weather (20-25°C). The longest survival recorded during other laboratory rearings of females was 11 days. All individuals became lethargic and increasingly unresponsive 1-2 days prior to dying, and did not oviposit during this period, despite still having mature eggs in their ovaries.

Clearly, the availability of honey almost doubled the lifespan of adult females (paired $t=9.804$, $df=31$, $p<0.001$). Their fecundity was probably thereby also increased, although this was not tested directly (see Section 3.5, Discussion).

3.5 Discussion

The shape of the egg of *T. venatoria* is similar to that of other species of trigonalyids which oviposit into the mesophyll, in that the ventral surface is round and smooth. In species that oviposit onto the leaf surface, the ventral surface of the egg is flat; it has been suggested that this difference could result from the egg's state of hydration rather than from the intrinsic structure of the chorion (Rodd, 1951). The eggs of *T. venatoria* (or of any other trigonalyid) do not, however, possess a pedicel. Such a structure is common in the eggs of many parasitic Hymenoptera (Hinton, 1981), and in eggs which absorb moisture from leaf tissue (Byrne *et al.*, 1990). The surface sculpturing is similar to that found in other species of *Taeniogonalos* (Rodd, 1951), and its structure may be such as to allow longitudinal cracking of the chorion along the surface ridges, without crushing the larva within, when the egg is subjected to the mandibular action of the host.

A combination of chemical (saline/acid/enzyme) and physical (needle manipulation) stimuli was most effective in causing eclosion, supporting the conclusions of Clausen (1931) for *P. thwaitesi* and *P. maga* (see Section 3.2.1). In contrast to the acid solution found to be effective in this study, Clausen used an alkaline solution presumably reflecting the gut conditions within a phytophagous lepidopteran host. In *Gonia cinerascens* Rond (Tachinidae; Goniinae) which has a similar method of host parasitisation, trypsin in alkaline solution has been shown to induce eclosion; its action was also enhanced by mechanical stimuli (Mellini and Campadelli 1978). There is no obvious reason for the lack of effectiveness of the gut and salivary homogenate as an inducer of eclosion, since this solution and sawfly gut contents have a similar pH. It is reasonable to suspect that eclosion percentages *in vivo* are far greater than those obtained *in vitro* in this study, in which the highest eclosion percentage for any replicate was only 17% (treatment 4).

The egg survival of 5 months demonstrated in this study is the longest documented for any trigonalyid, although members of the genus *Bareogonalos* are thought to have overwintering eggs which may remain viable for longer than this (see Section 3.2.3). Given the

method of parasitisation, it is not surprising that the eggs are able to survive for a long time in the field.

The development of *T. venatoria* is consistent with that described for other trigonalyids; there are five instars, and the larvae become ectoparasitic in the fourth instar.

Although first instar larvae could not be located in the gut or haemolymph, the setose larvae appear adapted to gut penetration, as are those of *P. maga* and *S. debilis* (Clausen, 1931). As third instar larvae were found free in the haemolymph, and as Clausen (1931) recovered first instar larvae of a different species from the haemolymph of papilionid and sawfly larvae, there is no reason to doubt that first instar larvae *T. venatoria* penetrate through the intestinal wall of their host. The occurrence of third instar larvae predominantly in the host prepupae, and the emergence of fourth instar larvae exclusively from the pupae, supports the generally held opinion that development beyond the first instar larva is delayed until the host is near pupation (Clausen, 1940; Riek, 1962b; Yamane, 1973), possibly in response to hormonal changes in the host (Beckage, 1985). This delay ensures host-parasitoid synchronisation in the ensuing generation (see below). The large number of third instar larva headcapsules recovered from individual host pupae indicate that superparasitism is common; this is not surprising considering that adult female *T. venatoria* oviposit several eggs along the same leaf margin. Because a solitary adult emerges from the host, it is likely that superparasites are eliminated in the third instar by combatative or parasitic cannibalism as suggested by Clausen (1929). However, enough host nutrients remain to support the development of a second adult (as demonstrated in host pupae removed from their cocoons), and these resources are normally wasted.

Only larvae acting as primary parasitoids were studies, as casual observations had indicated that larvae of *T. venatoria* acting hyperparasitically could be considerably smaller, particularly when parasitising *Frogattimya* spp. Such larvae invariably also gave rise to small adults. Yamane and Yamane (1975) report a similar variation in the size of *Bareogonalos huisuni* Yamane and Yamane developing on different casts of *Vespa*.

The cumulative phenology presented in Fig. 3.4 represents a reliable 'average' phenology, because the presence of the various stages was recorded during a 4 year period, which included 4 complete emergence seasons. It is deficient, however, in that it does not allow development to be correlated with climatic data.

It can be seen that, as a result of larval development in *T. venatoria* being delayed until host pupation, the emergence of host and parasitoid is synchronised, with *T. venatoria* following *P. dorsalis* by about two weeks. Consequently, maturing eggpods and actively feeding first and second instar *P. dorsalis* larvae are already on the foliage onto which emerging *T. venatoria* will oviposit. The time during which the eggs of *T. venatoria* must survive on foliage before being eaten is thereby minimised, and despite some eggs remaining viable for up to 5 months, overall egg mortality is thereby reduced (see Fig. 3.3). A similar synchronisation has been described for *P. thwaitesi* and its hosts in India (Clausen, 1929). This hyperparasitic trigonalyid emerges 1-12 days after the ichneumonid primary parasitoid, thereby allowing the ichneumonid time to parasitise the phytophagous lepidopteran host before the latter consumes the trigonalyid eggs.

The larval maturation time of *P. dorsalis* is variable (16-30 weeks), presumably because of variations in climate and resource availability. The length of time *T. venatoria* spends in the first instar is therefore also variable. However, once larval development of *T. venatoria* resumes within *P. dorsalis* pupae, the length of time spent in each instar appears to be fixed. Host regulation does not appear to affect diapause or the resumption of development in the larvae of *T. venatoria*, because the peak time for pupation in unparasitised sawflies is the same as that for sawflies parasitised by trigonalyids (not formally quantified). Such a lack of host regulation is consistent with the resumption of trigonalyid larval development being dependent on hormonal changes in the host, as suggested earlier. As a result, trigonalyids undergo prolonged diapause within their hosts (see chapter 6), and the longest previously recorded diapause in any trigonalyid is 2 years for *T. venatoria* in *Perga affinis* Kirby from inland N.S.W. and the A.C.T. (Carne, 1969). A new record of 3 years was established in this study for the same species in *P. dorsalis.*

Male *T. venatoria* are extremely rare, and it is therefore assumed that reproduction is largely by thelytokous parthenogensis. The male lacks sternal projections (used in the female to stabilise the abdomen during oviposition), and the presence of tyloids on the antennae (See Chapter 1) tends to implicate the involvement of sex pheromones in mating. Although all mating attempts failed in captivity, the male appears to be reproductively functional, and facultative mating may take place in the field. Thelytokous parthenogenesis is a derived condition found frequently among the parasitic hymenoptera (Hung, 1988), and it has been suggested that a reversion to male-dependent reproduction during environmentally adverse conditions may be adaptively advantageous in some species (Gordh and Lacey, 1976). It is well established, for example, that many thelytokously parthenogentic species will produce males if reared at high temperatures, and the underlying mechanism involves temperature sensitive chromosomal incompatibility microorganisms which distort the sex ratio (Werren, 1990). It is therefore interesting to note that the few male *T. venatoria* recovered in this study and by Carne come from colder areas of the host's distribution (i.e. Naracoorte, S.A., Brindabella Ranges, N.S.W.; see Chapter 1 and Section 2.3); this is the reverse effect of temperature to that describe for other temperature induced males (Werren, 1990).

Upon emergence, females already carry 6500 eggs. Assuming a life span of about 6 days (as for fed females), an oviposition rate of over 1000 eggs per day is likely. Since a female can live for up to to 11 days, and since new eggs develop during a female's lifetime, *T. venatoria* is likely to have a fecundity of over 10,000. This is an extermely high fecundity as compared to other parasitic hymenoptera, but is similar to egg counts made in other species of trigonalyids. Clausen (1929, 1931), for example, counted 8,218 eggs on dissection of *P. thwaitesi*, and 10,641 eggs oviposited by the same species in 14 days. He also records this species as ovipositing 4,376 eggs in one day! With the method by which trigonalyids parasitise their hosts, such a high fecundity may be expected.

When oviposition is limited, as in captivity, it is surprising to find females dying with several thousand eggs still in their ovaries, with no evidence of egg reabsorption. One might expect that such

reabsorption under adverse conditions would prolong life, thereby increasing the total number of ovipositions despite the partial loss of egg numbers.

Adults of both sexes clearly take food, and feeding almost doubled the longevity of females. Adult feeding has also been described in other species (Clausen, 1929; Townes, 1956), and it is therefore likely that all trigonalyids feed as adults. Comments in the literature that some species do not feed as adults (Oehlke, 1983b) maybe based on insufficient observations.

Whether or not food intake affects egg development in *T. venatoria* was not directly tested in this study. However, it was established that honey intake prolongs life span, and that additional eggs develop during a female's lifetime. It is therefore likely that food intake enhances egg development, at least indirectly.

CHAPTER 4

THE HOST RELATIONSHIPS OF *TAENIOGONALOS VENATORIA*.

CHAPTER 4

Host relationships of *Taeniogonalos venatoria* Riek

4.1 Introduction

It has been generally accepted that trigonalyids act as obligate secondary parasitoids (Clausen, 1940; Gauld and Bolton, 1988). The phytophagous host may be a lepidopteran, symphytan or nematoceran larva, and the intermediate host may be an ichneumonoid or tachinid primary parasitoid, or the larva of a predatory vespoid (Weinstein and Austin, 1991: Appendix 1).

The only record of primary parasitism in trigonalyids is that of Raff (1934), who concluded on the basis of good, but nevertheless circumstantial evidence, that *Taeniogonalos maculata* (Smith) was a primary parasitoid of sawflies in the genus *Perga.* It was the aim of this study to determine experimentally if *Taeniogonalos venatoria* Riek could develop as a primary parasitoid on *Perga dorsalis* Leach, or whether it too was an obligate secondary parasitoid.

4.2 Materials and Methods

Mature eggpods of *P. dorsalis* were collected from the field from *Eucalyptus leucoxylon* and *E. camaldulensis* near Adelaide in April 1988. The eggs were hatched and the larvae reared on potted *E. camaldulensis* in an insect-proof nethouse. In their second instar, the larvae were fed on *E. camaldulensis* foliage upon which captive female *T. venatoria* had oviposited a few weeks previously. This was therefore the only parasitoid to which the sawfly larvae were exposed at any time. Soil was provided in wooden boxes in October 1988, into which more than 200 larvae duly entered, and formed their cocoons normally. In early March 1989, 50 of the cocoons were dissected, and their contents were carefully placed in 5ml perspex vials. The latter had perforated caps to allow some exchange of air, and transparent sides through which development could be observed. One hundred and fifty of the remaining cocoons were retained in cages and emergences were recorded during March and April 1989.

Four hundred field collected cocoons were also dissected, their contents placed in perspex vials, and development and emergences were recorded during March and April 1989.

Both the rearing and collection of cocoons was repeated for another readily available species of pergid, *Pergagrapta condei* Benson. Emergence statistics were also collected from approximately 2000 *P. dorsalis* cocoons which did not form part of this experiment, to assess the percent parasitism attributable to each of the parasitoids of *P. dorsalis.* Host records for trigonalyids worldwide were compiled from the literature to compare the host relationships of *T. venatoria* to those of other species.

4.3 Results

The emergences of trigonalyids from both laboratory reared and field collected *P. dorsalis* cocoons are given in Table 4.1.

The emergences of adult female *T. venatoria* from the cocoons of *P. dorsalis* larvae which had been exposed *only* to this parasitoid confirms experimentally that *T. venatoria* can complete development as a primary parasitoid. Its emergence from *Westwoodia* cocoons and *Froggatimyia* puparia demonstrates the ability of *T. venatoria* to complete development as a secondary parasite in the same phytophagus host species, viz. *P. dorsalis*. *T. venatoria* is therefore a facultative hyperparasitoid of *P. dorsalis*. The emergences from the similarly treated cocoons of *Pergaprapta condei* are given in Table 4.2 (not quantified). An identical complex of emergences was observed from the cocoons of this species, and *T. venatoria* is therefore also a facultative hyperparasitoid of *Pg. condei*.

These are the first experimentally confirmed records of (1) a trigonalyid developing as a primary parasitoid in the same host that originally consumed the trigonalyid eggs, and (2) a trigonalyid completing development as either a primary or secondary parasitoid within the same host species.

The emergences from the field collected cocoons indicate that approximately 85% of *T. venatoria* develop as primary parasitoids, 11% as hyperparasitoids in ichneumonids, and only 4% as hyperparasitoids in tachinids (Table 4.1). Emergences from approximately 2000 *P. dorsalis* cocoons which did not form part of this experiment indicate that *T. venatoria* accounts for 28% of total emergences from *P. dorsalis* cocoons in the Adelaide region, and *P. dorsalis, Froggatimyia* spp. and *Westwoodia* sp. account for 39%, 32% and 1% of total emergences from *P. dorsalis* cocoons respectively (Table 4.3).

Host records for trigonalyids worldwide, including those obtained for *T. venatoria* in the present study, are given in Weinstein and Austin 1991 (Appendix 1). The major host groups of individual subfamilies and genera of world trigonalyids are summarised in Table 4.4, and the possible host relationships are summarised in Figure 4.1.

TABLE 4.1

Total emergences of T. venatoria from both laboratory reared and field collected P. dorsalis cocoons.

Number and type of cocoons	No. of adult female T. venatoria emerging			Other emergences of adults			
	No evidence of other parasitoids in same cocoon	Emergence from cocoon of Westwoodia sp.	Emergence from cocoon of Froggatimyia sp.	P. dorsalis	Froggatimyia sp. (Tach.)	Westwoodia sp. (Ich.)	No emergence (Death or diapause)
From cocoons of P. dorsalis exposed only to T. vevatoria:							
50 (Plastic vials)	2	0	0	Yes	No	No	Yes
150 (Emergence cages)	7	0	0	Yes	No	No	Yes
From field collected cocoons of P. dorsalis:							
400 (Plastic vials)	44	6	2	Yes	Yes	Yes	Yes
% of emerging T. venatoria:	85%	11%	4%				

Table 4.2

Emergences of T. venatoria from both laboratory reared and field collected Pergagrapta condei cocoons.

Type of cocoons	Emergence of adult female T. venatoria			Other emergences of adults			
	No evidence of other parasitoids in same cocoon	Emergence from cocoon of Westwoodia sp.	Emergence from cocoon of Froggatimyia sp.	Pg. condei	Froggatimyia sp. (Tach.)	Westwoodia sp. (Ich.)	No emergence (Death or diapause)
From cocoons of Pg. condei exposed only to T. vevatoria:							
In plastic vials	Yes	No	No	Yes	No	No	Yes
From field collected cocoons of Pg. condei:							
In plastic vials	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 4.3

The type and number of insects emerging from 2000 P. dorsalis cocoons collected in the Adelaide region. The percentage emergence is given, and the percentage parasitism has been calculated from the rates of hyperparasitism given in Table 4.1.

	P. dorsalis	T. venatoria	Froggatimyia sp.	Westwoodia sp.
No. of emergences	791	560	640	19
Percentage of emergences	39%	28%	32%	1%
Percentage parasitism		28%	35%	2%

Table 4.4. The number of species and subspecies, known distribution and major primary and secondary host groups of trigonalyid genera (P = Palaearctic; NA = Nearctic; NT = Neotropical; O = Oriental; E = Ethiopian; A = Australian).

Subfamily	Genus	No. of species and subspecies	Distribution	Major Primary Host Group(s)	Major Secondary Host Group(s)
Bareogonaloinae	*Bareogonalos*	4	NA, NT, O, P	? Lepidoptera	Vespidae
Discheinae	*Discenea*	6	E	Lepidoptera	unknown
	Lycagastroides	3	E	Lepidoptera	unknown
Lycogastrinae	*Ichnogonalos*	1	O	unknown	unknown
	Labidogonalos	4	NT	unknown	unknown
	Lycogaster	14	NA, NT, O	Lepidoptera	Ichneumonidae
				? Lepidoptera	Vespidae
				? Lepidoptera	Eumenidae
	Lycogonalos	1	O	unknown	unknown
	Stygnogonalos	1	E	unknown	unknown
	Taeniogonalos	12	A, NT, O	Pergidae	Tachinidae
				Pergidae	Ichneumonidae
				Lepidoptera	Ichneumonidae
				? Lepidoptera	Vespidae
	Tapinogonalos	4	E	unknown	unknown
Nomadininae	*Bakeronymus*	2	O	? Lepidoptera	Vespidae
	Nomadina	5	NT	? Lepidoptera	Vespidae
	Pseudonomadina	1	O	? Lepidoptera	Vespidae
Seminotinae	*Seminota*	7	NT	? Lepidoptera	Vespidae
	Xanthogonalos	3	NT	unknown	unknown
Trigonalyinae	*Mimelogonalos*	6	A	unknown	unknown
	Nanogonalos	4	NT, P, O	unknown	unknown
	Orthogonalys	9	E, NA, NT, O	Lepidoptera	Tachinidae
	Poecilogonalos	18	NA, P, O	Lepidoptera	Tachinidae
				Lepidoptera	Ichneumonidae
				Lepidoptera	Braconidae
				Symphyta	unknown
				Tipulidae	Tachinidae
	Satogonalos	3	P	unknown	unknown
	Trigonalis	2	P, O	? Lepidoptera	Vespidae
				Lepidoptera	Ichneumonidae
				Diprionidae	unknown
	Trigonalys	2	NT	unknown	unknown

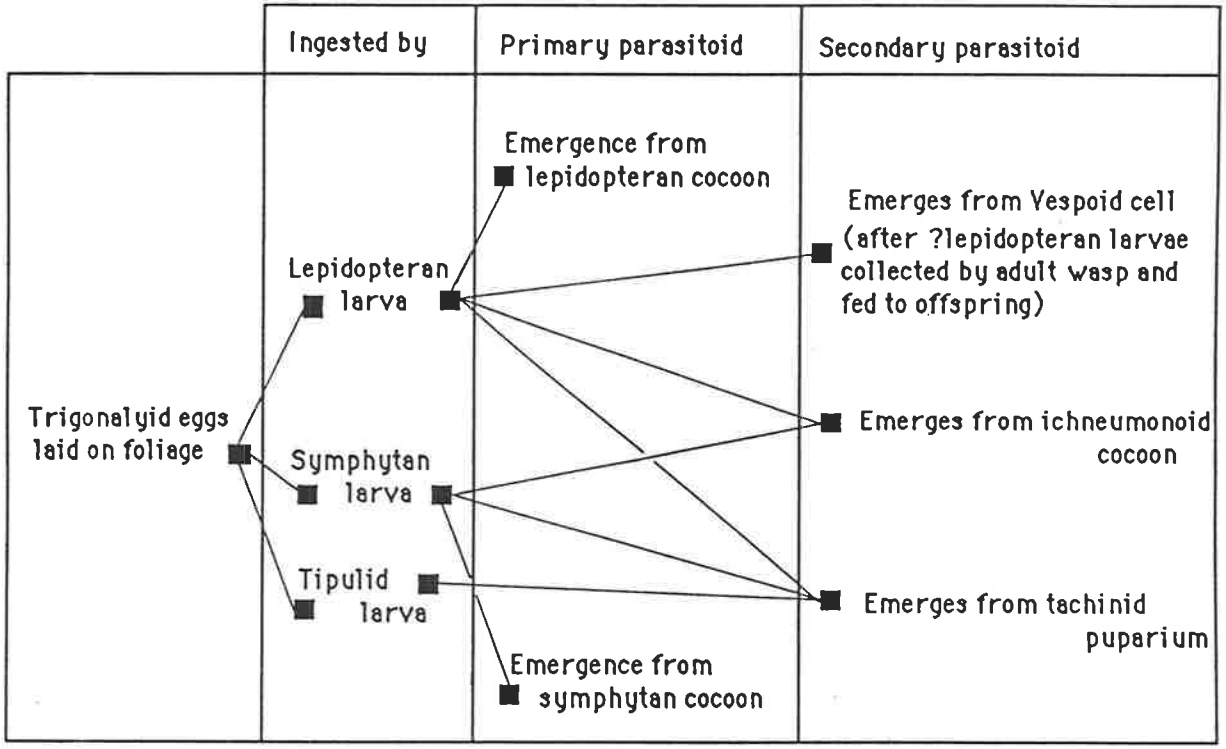


Figure 4.1.
Summary diagram of the host relationships of trigonalyid wasps.

4.4 Discussion

Prior to this study, the only evidence of trigonalyids acting as primary parasitoids was that of Raff (1934). Raff's evidence is based on dissections of pergid cocoons: She could find no exuviae, cocoons or puparia of parasitoids other than *T. maculata* within the pergid cocoons, and concluded that *T. maculata* was a primary parasitoid of *Perga.* She did not, however, take into account that the ectoparasitic final instar trigonalyid larva often destroys the remains of the host by crushing and chewing the tissues (Cooper, 1954), and that it may therefore be impossible to identify the remains of other parasitoids within the same host. Although the ectophagous larva of *T. venatoria* would occur within an ichneumonid cocoon or tachnid puparium if these parasitoids were present in the same host, the possibility nevertheless remained that the presence of other parasitoid larvae was essential in the earlier stages of development of *T. venatoria* for physiological reasons such as to overcome host immune defences. All traces of such larvae may have been destroyed by the final instar trigonalyid larva before it pupated in the sawfly cocoon. This experiment demonstrates that the presence of other parasitoid larvae is not essential to the developemt of *T. venatoria.*

The fact that 85% of adult *T. venatoria* developed as primary parasitoids indicates that this is by far the most successful mode of parasitism for this species. Eleven percent of adult *T. venatoria* developed as hyperparasitoids of *Westwoodia,* which is a very high percentage considering the small number of *P. dorsalis* larvae parasitised by *Westwoodia* (about 2% - Table 4.3). Development on *Froggatimyia* spp. was least successful, and accounted for only 4% of emergences of *T. venatoria*, despite the fact that about 35% of *P. dorsalis* contained the larvae of *Froggatimyia* spp. (Table 4.3). Host size is probably a significant factor in determining the success of parasitisation, with *Froggatimyia* spp. puparia containing by far the smallest amount of resources for the developing larvae of *T. venatoria.*

From the summary host records of trigonalyids world-wide given in Table 4.4, it can be seen that trigonalyids have been reared from three orders of primary hosts, i.e. Hymenoptera (Diprionidae, Pergidae),

Lepidoptera (several families) and Diptera (Tipulidae), and two orders of secondary hosts, i.e. Hymenoptera (Ichneumonidae, Braconidae, Vespidae, Eumenidae) and Diptera (Tachinidae). Vespids are listed as secondary hosts because they must consume a primary host (lepidopteran larva) to become parasitised - See Figure 4.1. Vespids are apparently the most common secondary host group of trigonalyids, but the data on which this conclusion is based are probably heavily biased because of the detailed attention given to the Vespidae and their ease of collection, compared with other potential host groups. For individual species of trigonalyids (see Appendix 1), probably the most enigmatic and divergent host record is that for *Poecilogonalos costalis* (Cresson), which has been reared from a nematoceran primary host (*Tipula* sp.) via a tachinid secondary host (Gelhaus, 1987). There are also isolated records of trigonalyids having been reared from eumenid cells (Cooper, 1954) and from braconid cocoons via lepidopteran primary hosts. The young larvae of *Poecilogonalos thwaitesi* (Westwood) have even been reported to parasitise older conspecific larvae feeding on the ichneumonid *Henicospilus* sp. (Clausen, 1929), making this trigonalyid a facultative tertiary parasitoid. Some subfamilies and genera of world trigonalyidae (Table 4.4) demonstrate restricted host relationships for at least some taxa, even though the available data are limited: The Bareogonalinae (*Bareogonalos*), Nomadininae (*Nomadina, Pseudonomadina*) and Seminotinae (*Seminota*) have only been associated with vespid secondary hosts, while the Disceninae apparently exploit only lepidopteran primary hosts. At the generic level there are four taxa of trigonalyids (*Lycogaster, Poecilogonalos, Taeniogonalos* and *Trigonalis*) that can be considered to be moderately polyphagous, because they have been reared from diverse primary and secondary host groups. However, the remaining genera have no recorded host data or there are too few records to draw any particular conclusions. At the species level, several trigonalyids have been reared from several different primary and secondary hosts. They include *Bareogonalos jezoensis* (Uchida), *Lycogaster pullata* (Shuckard), *Poecilogonalos costalis* (Cresson) (Appendix 1) and *T. venatoria* (Tables 4.1, 4.2). *B. jezoensis* has been reared from 12 secondary hosts, but all are species of *Vespa* or *Vespula*. *L. pullata* has been reared from diverse secondary host families, but like *B.*

jezoensis is probably restricted to lepidopteran primary hosts. *P. costalis and Trigonalis hahnii* Spinola, on the other hand, have been reared from diverse primary (Lepidoptera, Tipulidae, Diprionidae) and secondary (Tachinidae, Ichneumonidae, Vespidae) host groups. Interestingly, trigonalyids have not been recorded from leaf-feeding beetle larvae.

In summary, trigonalyids have been recorded as primary parasitoids in the present study (Tables 4.1, 4.3); as secondary parasitoids (several authors - see Appendix 1); and as tertiary parasitoids (Clausen, 1929 and Chapter 3, Larval development, third instar). There are also very diverse host records, even within the same species, and in this study *T. venatoria* was shown to act as a facultative hyperparasite. These host records provide support for the commonly held opinion that members of the Trigonalyidae, unlike other parasitoids, do not demonstrate strong host specificity (Cooper, 1954; Gelhaus 1987). It is likely that trigonalyids, because of their unique host selection behaviour and oviposition strategy (Chapter 5) are more dependent on host biology than on host phylogeny as a determinant of successful parasitism. Almost any host with appropriate feeding behaviour and physiognomy appears susceptible to parasitism by trigonalyids. For example, because of the way in which the egg chorion needs to be broken (Chapter 3.4.1), it is likely that host age (manible size) and feeding method (mandible shape) has a greater effect on host acceptability than does host phylogeny. The host range of trigonalyids appears wider than that of any other hymenopteran parasitoid (Askew, 1971; Gauld and Bolton 1988), and trigonalyids are therefore probably the most r- selected of hymenopteran parasotoids.

CHAPTER 5

THE HOST SELECTION BEHAVIOUR OF *TAENIOGONALOS VENATORIA*.

CHAPTER 5

The host selection behaviour of *Taeniogonalos venatoria*

5.1 Introduction

The process by which a parasitoid successfully parasitises its host involves a series of five steps (Vinson 1976, 1985). Based on the early research of Salt (1935, 1937) and Flanders (1937, 1947), Doutt (1959) described the first four of these as: (a) Host habitat location, (b) Host location, (c) Host acceptance, and (d) Host suitability. Vinson (1975) added (e) Host regulation.

The first step involves the long range location of a region (habitat) likely to contain the host species. It is necessary in parasitoids which emerge distantly from a host population, either because of the spatial/temporal clumping of the latter, or as a result of parasitoid dispersal during a preoviposition period. An example is the location of carrion by *Nasonia vitripennis* (Walker) (Pteromalidae), which is a parasitoid of dipteran maggots; carrion is located by volatile chemical cues which are not dependent upon the presence of the host (Laing, 1937).

The second step, host location, involves the actual detection of an individual host. It is an obvious prerequisite to any oviposition into or onto the host. *Cardiochiles nigriceps* Viereck (Braconidae), for example, locates *Heliothis* larvae by the latter's mandibular gland secretion, a non-volatile contact chemical (Vinson *et al.*, 1975).

The third step involves the acceptance or rejection of a host for oviposition on the basis of cues perceived by the female parasite in the perioviposition period. The parasitoid may for example detect a

marker pheromone on the host, left there by a conspecific female at oviposition, which may render the host unacceptable for further oviposition (host discrimination) (Anderson, 1988; Hofsvang, 1990).

Host suitability refers to those aspects of the host (not detected at oviposition) which influence the survival of the parasitioid after oviposition; nutritional content and concurrent disease are examples of such factors (Vinson and Iwantsch, 1980).

The fifth and final step is one whereby the parasitoid's egg, larva, or accompanying inoccula regulate the physiology and/or behaviour of the host to optimise the parasitoid's own survival. A good example of host regulation is the host immunosupression induced by symbiotic viruses, which are injected with the eggs of some parasitoids (Stoltz and Vinson, 1979).

The first three of these steps involve active behavioural processes by the ovipositing female, and Vinson (1976) groups them together as 'host selection'. This is the sense in which 'host selection' is used here, although Pak restricts the term to Vinson's third stage (host acceptance) (Pak *et al.* 1986).

A few hymenopteran parasitoids oviposit on foliage, for example the Perilampidae and Eucharitidae (see Askew, 1971, Gauld and Bolton 1988), and thereby leave the emergent planidial first instar larvae to complete host selection by locating and accepting a host. Trigonalyids also oviposit on foliage, but their eggs do not eclose unless ingested by a suitable host (See Chapter 3). Among the Hymenoptera, this is a unique method of parasitising a host, and some tachinid flies (Goniinae) are the only other parasitoids known to employ the method (Askew, 1971). In *T. venatoria*, therefore, host selection is largely replaced by the location and acceptance of foliage for oviposition. The cues likely to influence the trigonalyid *T. venatoria* in its search for oviposition sites, such as the presence of host larvae, have not previously been studied, and are therefore quantified in this chapter.

5.2 Materials and Methods: Insects

Adult *T. venatoria* were collected during April 1988 from cocoons of the sawfly *P. dorsalis* held in emergence cages. Cocoons of both field collected and laboratory-reared *P. dorsalis* were used. Adults were collected within 1h of emergence, and were placed individually in clean 20ml plastic holding vials. No trigonalyid spent more than 30 min in a holding vial before being used in an experiment, except for those used to determine oviposition rates (see 5.5.1.2). In the latter group, adults in vials were held at 4°C for varying periods of up to 24h to allow time for previously used *T. venatoria* to be cleared from the limited number of oviposition cages which were available. This refrigeration had no obvious effect on adult behaviour, with individuals resuming normal activities within 5 min of being placed at room temperature (20–25°C).

Experimental trigonalyids were used as either 'naïve unfed', 'naïve fed', or 'experienced' individuals. Naïve unfed individuals were used directly upon removal from their holding vials. Naïve fed individuals were allowed to feed freely upon a drop of honey in water, and were not used until they had ceased feeding of their own accord. Experienced individuals had been released into oviposition cages containing *Eucalyptus camaldulensis* foliage, upon which they were allowed to oviposit for 1 to 2 days before being used. These cages also contained actively feeding first and second instar *P. dorsalis* larvae and their frass, as well as water and honey, so as to allow exposure of the trigonalyids to any possible cues required to prime their behaviour (Drost *et al.*, 1986). Because of the limited availability of adult *T. venatoria*, naïve individuals were reused as experienced individuals after being used in experiments in which they were exposed to *E. camaldulensis* foliage. Such individuals were also released into oviposition cages, where they remained for 1 to 2 days before being reused.

5.3 Section 1. Host habitat location: Laboratory studies

5.3.1 Materials and Methods

The response of trigonalyids to olfactory cues was assessed in both a Y-tube olfactometer, a 4 armed "odourfield" olfactometer, and in a flight-tunnel.

5.3.1.1 The Y-tube olfactometer

The Y-tube olfactometer consisted of 0.75 cm diameter perspex pipe joined with silicone into the shape of a 20cm tall "Y". Air flow was created by joining a venturi pump on an adjacent tap to the base of the "Y" with silastic tubing. A small piece of gauze was placed at a join in the tubing to allow experimental animals to be inserted. The airflow in each arm of the "Y" was monitored with an airflowmeter (Fisher and Porter No. 1/1620G5/84), allowing the airflow to be balanced between the arms. The air intake into each arm was through a series of 3 sealed perspex vials, joined by silastic tubing as illustrated in Figure 5.1.1. The first vial filtered the air through activated charcoal, eliminating contaminating odours; the second contained a distilled water airlock, which humified the air and acted as a second filter; the third was replaceable and contained the odour source or control (empty vial). This vial also acted as a reservoir for retrieving experimental animals (see Figure 5.1.1). In any one replicate, one arm of the Y-tube was the treatment arm; the other was the control. Eight or 10 trigonalyids were released sequentially into the olfactometer, and the number choosing each arm was recorded. The odour source and control vials were reversed after each replicate.

Three odour sources were used: intact *E. camaldulensis* leaves; honey plus mechanically damaged *E. camaldulensis* leaves; and *E. camaldulensis* leaves plus first and second instar *P. dorsalis* larvae (which had been feeding actively upon the same). The intention was to expose naïve unfed, naïve fed and experienced trigonalyids to each of these 3 odour sources, thereby enabling an ANOVA to be performed to determine the effect of both odour source and trigonalyid type. Unfortunately, insufficient naïve unfed trigonalyids were available, and the exposure of naïve unfed trigonalyids to foliage with *P.*

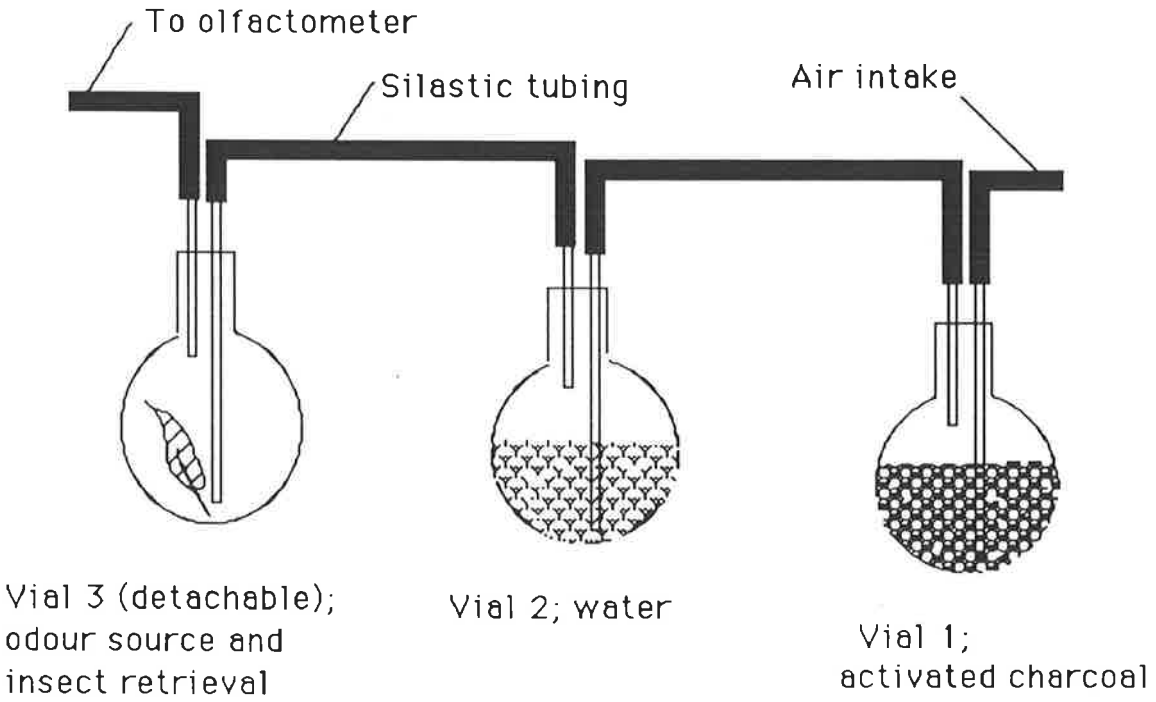


Figure 5.1.1

Three sealed perspex vials through which air was passed before entering each arm of the olfactometer. Air was filtered (vials 1 and 2), humidified (vial 2) and exposed to an odour source or control (vial 3).

dorsalis larvae was omitted. All other treatments were completed; naïve unfed, naïve fed, and experienced *T. venatoria* were exposed to the first two odour sources, and naïve fed and experienced trigonalyids were exposed to the third odour source.

Each experiment was carried out in a constant temperature room at 25°C, with standard overhead flourescent lighting. Light intensity did not differ between the arms, as measured by a light meter (0.075 micromole quanta photosynthetically activate radiation/m^2/s; Li'cor photon flux density meter), and all equipment (including the observer) was kept in a symmetrical position with respect to the "Y" tube. Because 8 individuals were used in some replicates and 10 individuals in others, the number selecting the arm with the odour source was converted to a figure out of 8, thereby giving each replicate an equivalent numerical weighting in the analysis. The influence of the type of treatment was analysed by one factor ANOVA and, where possible, the influence of the interaction between type of odour source and type of trigonalyid was analysed by 2 factor ANOVA.

5.3.1.2 The 4-armed "odourfield" olfactometer

The 4-armed "odourfield" olfactometer was constructed by Dr. G. Pak (Wageningen) during his visit to the Department in July 1986, and was similar to the one described by Vet *et al.* (1983). A square central arena is supplied with an air inlet and odour source in each corner, and is drained by a central suction hole which is also the point of insect introduction (Figure 5.1.2). Insects can enter, exit and re-enter 4 adjacent odourfields in the central arena before walking out through the corner in which they are finally counted.

The airflow was created by a venturi pump, balanced by a flowmeter in each arm, and passed through 3 vials before entering in each corner, as described for the Y-tube olfactometer (Figure 5.1.1). Only one odour source was used, namely *E. camaldulensis* leaves plus first and second instar *P. dorsalis* larvae (which had been feeding actively upon the same). This was placed in one corner only, with the other three corners acting as controls (empty vials). Naïve fed and experienced trigonalyids were exposed to this odour, and 12 or 16 were released sequentially in each replicate, depending on availability. Six replicates were run with each type of trigonalyid, and the number

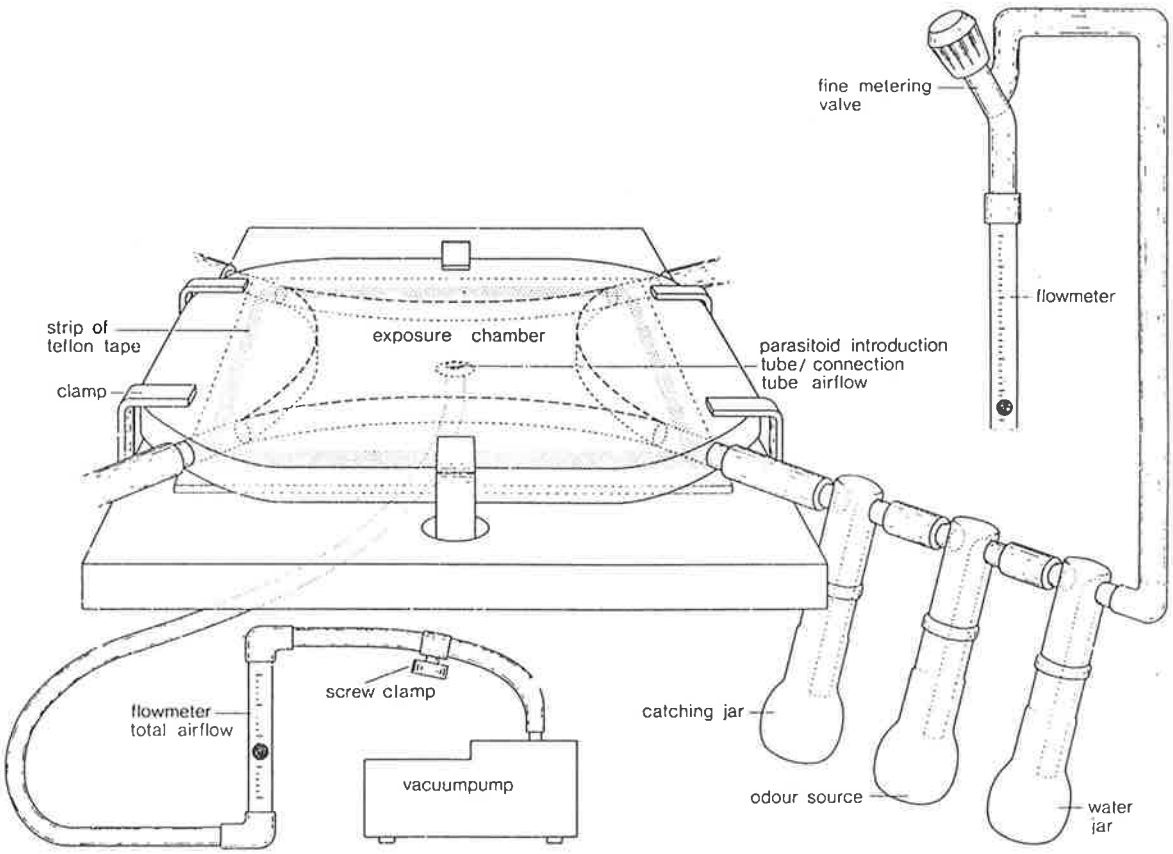


Figure 5.1.2
The 4-arm odour field olfactometer (from Vet *et al.* 1983). Air is drawn through the exposure chamber by a Venturi vacuum pump, attached to the central outflow/parasitoid introduction tube. The outflow from each arm through the exposure chamber is equalised by adjusting the flow meters at the 4 air inlets. Internal dimensions of chamber: height, 10mm; narrowest width, 110mm; inside diameter of air inlet tubes, 10mm.

choosing each arm was recorded. The odour source was moved clockwise through 90° after each replicate, and the arms were designated as follows; 1= odour source, 2= 90° clockwise from odour source, 3= opposite odour source, and 4= 90° anticlockwise from odour source. The temperature and lighting conditions were as described for the Y-tube olfactometer. The hypothesis that both naïve fed and experienced trigonalyids exit through a corner at random was tested by 2 factor ANOVA of trigonalyid type and arm number (odour source) on number of exits.

5.3.1.3 The flight-tunnel

The flight-tunnel was constructed by Dr. M.A. Keller (Waite Institute) in 1987, and is described by Keller (1990) in detail. A variable speed airflow is created in a large perspex tunnel by a fan, and is kept laminar by a panel of drinking straws lying parallel to the direction of flow at the air inlet. Utility ports allow the introduction of odour sources upwind and experimental insects downwind, and flight behaviour can be observed and/or videorecorded through the clear perspex walls. Due to a strong positive phototaxis demonstrated by trigonalyids in the tunnel under flourescent lighting, only diffuse incandescent background lighting was used. An activated charcoal filter at the air inlet ensured that the airstream in the tunnel, although recirculating, was free from contaminating odours. The tunnel was housed in a windowless insectary room at 25–27°C.

Naïve unfed, naïve fed, and experienced trigonalyids were challenged with 9 different odour sources at a windspeed of 41.3 cm/s, and two of these odour sources at 3 other windspeeds (18.0, 66.3 and 109.0cm/s) as set out in Appendix 4.3.1. The odour sources were as follows:

(A) Twig of 5-6 intact *E. camaldulensis* leaves (stalk in 50ml conical flask with water and cotton wool).

(B) as for A, leaves partially cut with scissors.

(C) as for A, leaves plus first and second instar *P. dorsalis* and feeding damage caused by same.

(D) as for A, twig also with flowers and a drop of honey.

(E) Petri dish of *P. dorsalis* frass.

(F) as for C, plus frass.

(G) 4-5 large drops of fresh meconium from newly emerged *P. dorsalis* females, in a petri dish.

(H) as for A, but with a *P. dorsalis* eggpod on one leaf, and meconium as for (G) underneath.

(I) as for A, with an unmated female *P. dorsalis* perched on one leaf.

The odour sources were placed 10cm from the upwind end of the flight chamber, on a clean plastic block which raised the source to the centre of the airstream. Windspeeds used were higher than those that have been used successfully with smaller parasitoids such as *Campoletis sonorensis* (Cameron) (Ichneumonidae) (5cm/s; Elzen *et al.*, 1986) or *Microplitis croceipes* (Cresson) (Braconidae) (23cm/s; Drost *et al.*, 1986). The relatively large *T. venatoria* (>1cm) were expected to be strong fliers, and this was confirmed by test flights in the tunnel. Six to 12 experimental trigonalyids were released sequentially in each replicate, and the number that contacted or alighted upon the odour source within 7sec was counted. The only 'flights' longer than 7sec were invariably interrupted by momentary alightings on the tunnel walls. Adults which flew directly to and alighted upon a tunnel wall in less than 1sec were captured and re-released. Alightings were therefore only counted for continuous flights of between 1 and 7sec, during which trigonalyids could reasonably be considered to have the opportunity to alter their flightpaths as a direct result of odours perceived in the airstream. Releases were effected by placing uncapped holding vials at the centre of the airstream on a clean plastic block, 10cm from the downwind end of the flight chamber. Trigonalyids would climb up the vials to the rim, and were timed with a manual stopwatch from takeoff.

An 'incidental' background rate of orientation to the 'odour source' was obtained by recording the outcome of 135 releases of experienced trigonalyids, with a disk of green paper on a wooden stick in place of the odour source (windspeed 41.3m/s). Before analysis, compensation was made for the different numbers of *T. venatoria* used in the various trials by converting the number of alightings in each trial to a percentage of the number of *T. venatoria* released in that trial. The

incidental rate of orientation was compared to the overall rate obtained using the 9 odour sources by a one-sample t-test. The odour sources were then grouped as (1)= A, (2)= C, and (3)= B, D, E, F, G, H and I, and the hypothesis was tested that orientation by *T. venatoria* at wind speeds between 18.0 and 66.3 cm/s is not significantly affected by trigonalyid type and odour source group.

5.3.2 Results

5.3.2.1 Y-Tube Olfactometer

The type of odour source, type of trigonalyid, and number of *T. venatoria* choosing each arm are given in Table 5.1. for each of the 2 replicates of each treatment. The percentages of *T. venatoria* choosing the arm with the odour source are given in Table 5.1, and the analyses (ANOVA) for testing response to the odour source are given in Appendices 5.1.1, 5.1.2 and 5.1.3. The arm selected was not affected by type of treatment (one factor ANOVA, P=0.29, Appendix 4.1.1). To check the significance of the interaction between odour source and trigonalyid type, the incomplete treatments (refer materials and methods) were omitted, and the remaining treatments were analysed by a 2 factor ANOVA. When treatments with naïve unfed trigonalyids were omitted (ANOVA, Appendix 4.1.2), the arm chosen was not affected by odour source (P=0.59), trigonalyid type (P=0.12), or interaction of the two (P=0.22). In particular, experienced trigonalyids did not choose the arm leading to feeding host larvae more frequently than would be expected by chance alone. When treatments with feeding host larvae were omitted (ANOVA, Appendix 4.1.3), the arm chosen was not affected by odour source (P=0.94), trigonalyid type (P=0.76), or interaction of the two (P=0.17). In particular, naïve unfed trigonalyids did not choose the arm leading to honey more frequently than would be expected by chance alone.

The results indicate that trigonalyids, regardless of experience, did not orient to the olfactory cues emanating from intact *E. camaldulensis* leaves, honey with mechanically damaged *E. camaldulensis* leaves, or *E. camaldulensis* leaves with first and second instar *P. dorsalis* larvae (feeding actively upon the same).

5.3.2.2 4-Arm Odourfield Olfactometer

Table 5.1

The odour source, type of trigonalyid and number (N) and percentage of T. venatoria choosing the arm of the Y-tube olfactometer with the odour source, in each of 2 replicates of each treatment. The mean percentage choosing the arm with the odour source is given for each treatment.

Odour source	Trigonalyid type	Replicate number	n	N	% to source	Mean % for treatment
Euc. only	Naïve unfed	1	10	5	50.0	
		2	8	3	37.5	43.75
	Naïve fed	1	10	5	50.0	
		2	10	4	40.0	45.00
	Experienced	1	10	6	60.0	
		2	10	6	60.0	60.00
with honey	Naïve unfed	1	10	6	60.0	
		2	10	5	50.0	55.00
	Naïve fed	1	8	3	37.5	
		2	10	6	60.0	48.75
	Experienced	1	8	3	37.5	
		2	10	4	50.0	43.75
with larvae	Naïve fed	1	8	3	37.5	
		2	10	4	40.0	38.75
	Experienced	1	10	5	50.0	
		2	8	5	62.5	56.25

The trigonalyid type, arm number and average number of trigonalyids out of 12 exiting through a corner are listed in Table 5.2, and the raw data are given in Appendix 4.2.1. The analysis of the data (ANOVA) is given in Appendix 4.2.2, and suggests that the corner through which *T. venatoria* exited was not affected by trigonalyid type (P=0.90), arm number (odour source) (P=0.54), or interaction of the two (P=0.38).

The results therefore suggest that trigonalyids, regardless of experience, did not orient to olfactory cues emanating from *E. camaldulensis* leaves with first and second instar *P. dorsalis* larvae (which had been feeding actively upon the same).

5.3.2.3 Flight-tunnel

The average percentage of naïve unfed, naïve fed and experienced trigonalyids orienting to the three odour source groups are given for windspeeds between 18.0 and 66.3 cm/s in Table 5.3, together with the background orientation rate. The raw data are given in Appendix 4.3.1, including the individual windspeeds, particular odour sources, and number of trigonalyids in each replicate. The analysis of the data is given in Appendix 4.3.2.

The rates of orientation to the odour source were very low for all treatments. In the treatments at windspeed 109.0 cm/s, no *T. venatoria* alighted on the odour source, presumably because this windspeed was too high to permit controlled landings upwind. These treatments were therefore omitted, and the remaining windspeeds (18.0, 41.3 and 66.3 cm/s) were pooled for the analysis.

The incidental background rate of orientation (3.7%) did not differ from the overall rate of orientation obtained using the 9 odour sources (one sample t-test; t=1.07, df=38, P=0.30). The ANOVA (Appendix 4.3.2.) suggested that the orientation rate of *T. venatoria* was not affected by trigonalyid type (P=0.45), odour source group (P=0.27), or interaction of the two (P=0.64).

The results indicate that trigonalyids, regardless of experience, do not respond to olfactory cues emanating from *E. camaldulensis* leaves (intact, damaged, or with *P. dorsalis* larvae or flowers), nor to the olfactory cues emanating from the eggs, larvae, adults, frass or meconium of *P. dorsalis*.

Table 5.2

The trigonalyid type, total number of trigonalyids tested (n) and average number of trigonalyids entering each arm of the odourfield olfactometer in a replicate of 12 individuals. The average number for all arms and replicates (Total av.) is also given.

Trigonalyid type	Number of replicates	n	Average number of trigs per rep. entering arm				Total av.
			Arm 1	Arm 2	Arm 3	Arm 4	
Naïve fed	6	72	3.54	2.21	2.62	3.62	3.00
Experienced	6	72	3.25	3.33	2.71	2.71	3.00

Table 5.3

The number (N) and percentage of 3 types of T. venatoria orienting to 3 groups of odour sources in a wind tunnel, pooled for windspeeds between 18.0 and 66.3cm/s. The background rate obtained with a paper odour source is given for comparison.

Odour group (see 5.3.1.3)	Naïve unfed trigs			Naïve fed trigs			Experienced trigs			Av. % for odour group
	n	N	%	n	N	%	n	N	%	
1	30	2	**6.7**	30	0	**0**	34	1	**2.8**	**3.1**
2	18	1	**5.6**	28	1	**3.3**	26	2	**6.7**	**5.2**
3	50	2	**2.4**	52	1	**2.4**	72	0	**0**	**1.6**
Av. % for trig type			**4.1**			**2.1**			**2.2**	**Overall 2.8**
Background							135	5	**3.7**	

5.4 Section 2. Host habitat location: Field studies

5.4.1 Materials and Methods

The responses of naïve fed *T. venatoria* to known host trees of *P. dorsalis* (*E. camaldulensis*, *E. citriodora, E. leucoxylon, E. microcarpa*) and non-host elm trees (*Ulmus* sp.) were recorded in the field. No actively feeding host larvae were present in the host trees at the time of the study, but host larvae had been noted in the trees during at least one of the 1986 or 1987 seasons.

Host trees of 3 different heights were selected at 3 different locations: 2m *E. eucoxylon* and *E. microcarpa*, in the North Adelaide parklands; 12m *E. camaldulensis*, by the Adelaide University Oval; and 20m *Eucalyptus citriodora,* along the Waite Institute main driveway. Ten metre elm trees were also selected for use as non-host trees (Waite Institute, main driveway). All trees selected were in a single, tight row at least 40m long, and had a semi-circular tree-free clearing of at least 300m radius on one side of the row (see Figure 5.1.3). Canopy density and colour were similar in all rows, but were not quantified. A line at right angles to the row was drawn from the centre of each row into the adjacent clearing. From a specific point along this line, each of 10 ± 3 naïve fed *T. venatoria* were released by placing an uncapped holding vial at the release point, with the observer then crouching or lying down several meters away until take off. The flight path of each insect was recorded as either terminating in the selected trees, or terminating elsewhere (including trigonalyids lost from sight). The procedure was repeated at points along the line at 5 or 10m intervals. As trigonalyids released at the extreme distances (10m, 60m) tended to behave similarly, longer series of trigonalyids were released from the more central distances (25-40m).

The data were compiled throughout April, and releases were made both morning and afternoon, at temperatures varying from 18 to 32°C, regardless of cloud cover or wind direction. Releases were not made if the wind was too strong for the trigonalyids to successfully undertake oriented flight. By running along underneath a trigonalyid,

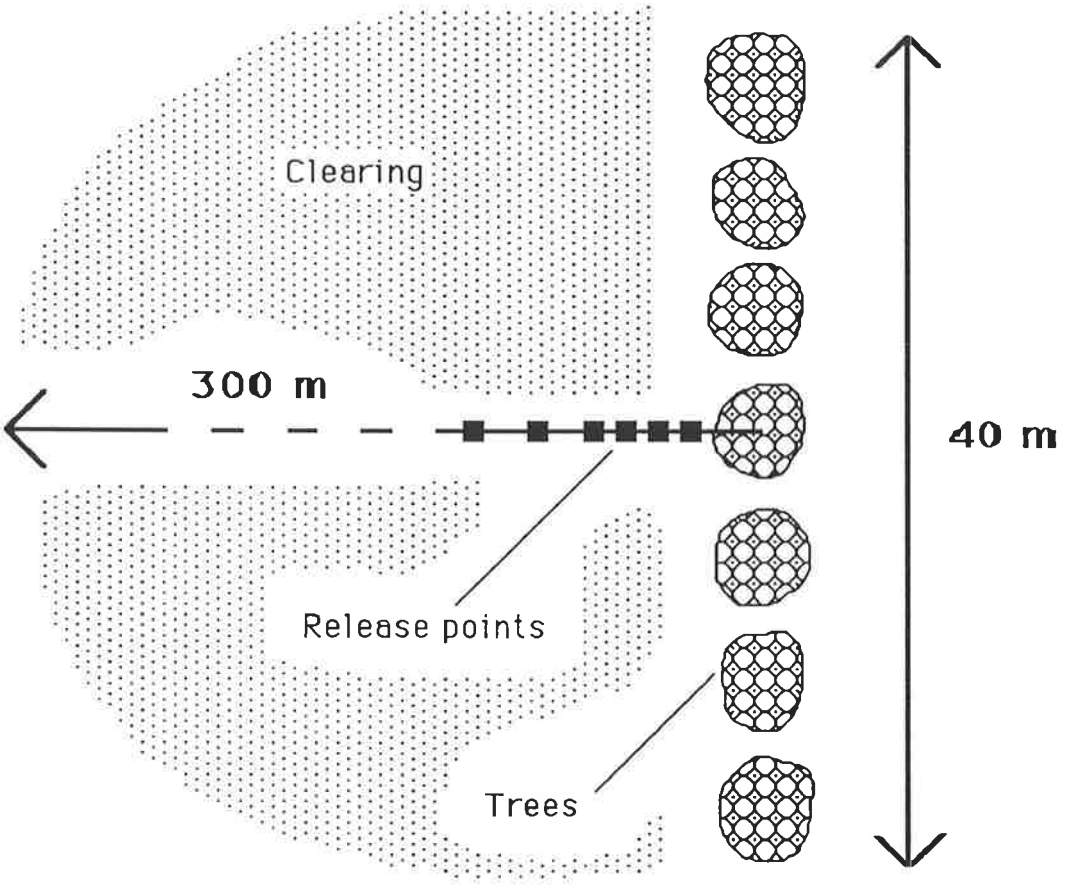


Figure 5.1.3

The layout of trees and adjacent clearing where release points were selected to study host habitat location in T. venatoria.

it was possible to confirm that it was pointed in the direction it was moving, regardless of whether it was moving up, down or across the wind. The possibility of counting trigonalyids which had simply been 'blown' to or from the trees was thereby eliminated.

For each class of tree, the percentage of trigonalyids landing in the trees was plotted against the distance from the trees at which the trigonalyids were released.

5.4.2 Results

For each class of tree, the number and percentage of trigonalyids which flew to the trees from each release point are listed in Appendix 4.4. Each release point is given both as a distance from the base of the closest tree, and as an angle subtended by a line joining the release point to the top of the closest tree.

A number of different plots were tried to determine a simple relationship between some property of the trees and the percentage of trigonalyids flying to the trees. Initially, for each class of tree, the percentage of trigonalyids flying to the trees was plotted against the distance from the trees (Figure 5.2.1). When released close to the trees, about 90% of trigonalyids flew to the trees, whereas only about 30% did so when the trees were more than 40m away. The percentages dropped quite sharply at some central distance ranging from about 20m for the 2m trees to 40m for the 20m trees. The distribution of points for the non-host elm tree (10m) did not appear to differ from that of the eucalypts. The data suggest that a family of sigmoidal curves may provide the best representation of the relationship between percentage response and distance from the trees.

When the probit of percentage response is plotted against the distance from the trees, straight lines can be fitted to the data, as may be expected (Figure 5.2.2). For three of the four classes of trees (2m, 10m, and 12m), R^2 values above 0.75 indicate a significant negative linear regression of probit percentage response on distance from the trees ($P<0.05$). Some of the regressions are more significant when x is expressed as the square root of the distance from the trees (Figure 5.2.3). From each of these regressions, the distance at which 50%

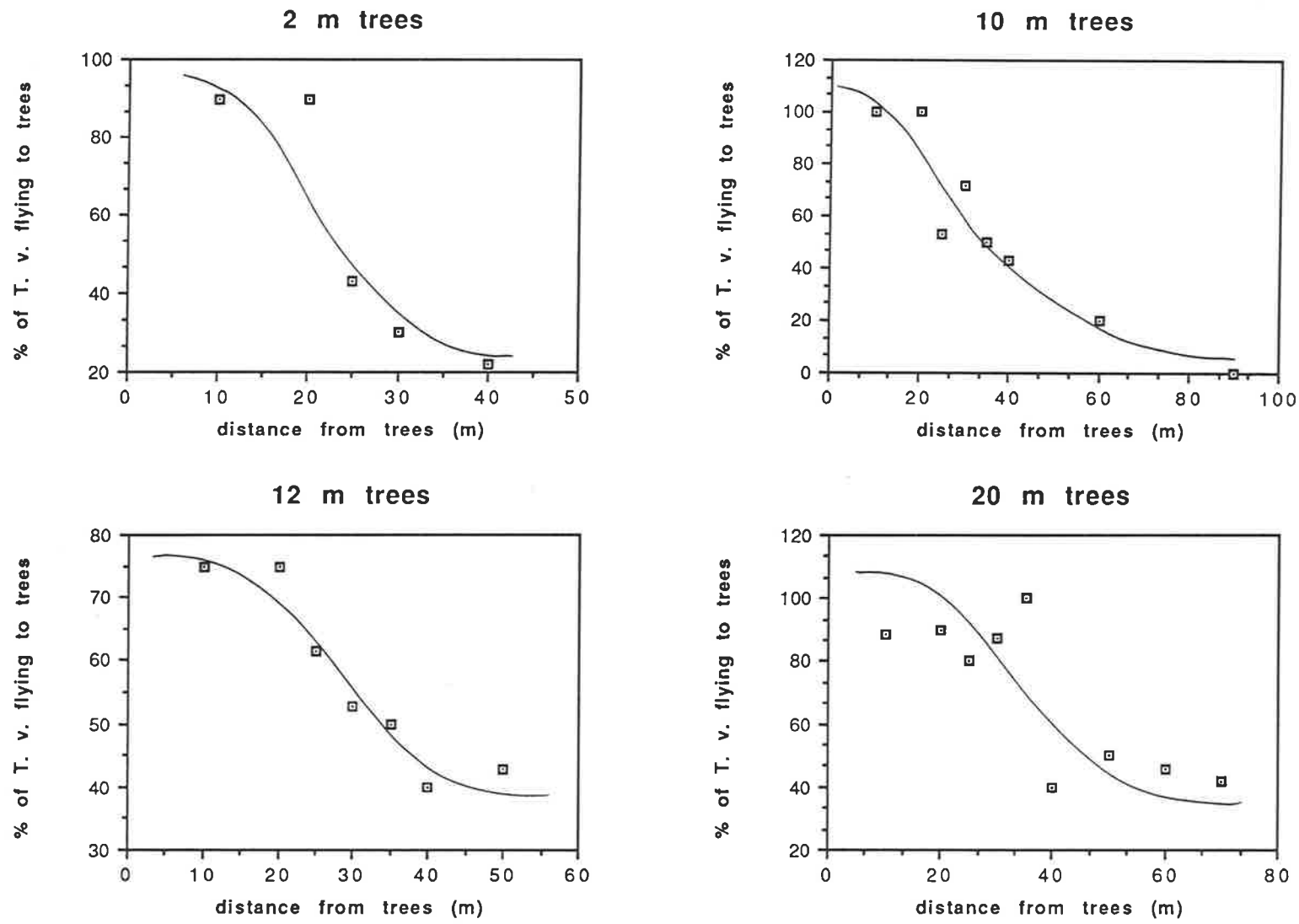


Figure 5.2.1. The percentage of trigonalyids flying to trees in relation to the distance from the trees, for each height class of tree seperately.

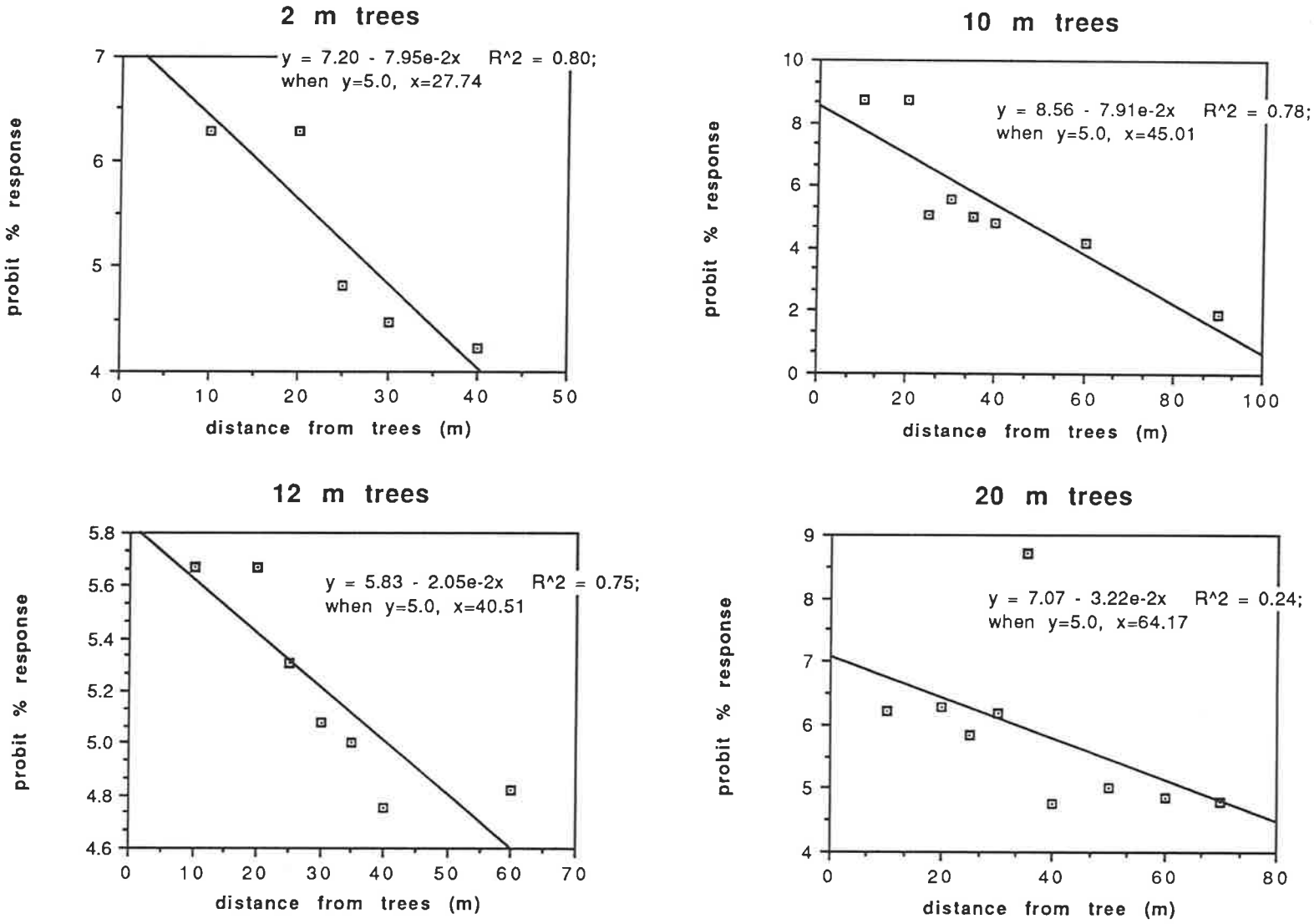


Figure 5.2.2. The probit of the percentage of trigonalyids flying to trees in relation to the distance from the trees, for each height class of tree seperately.

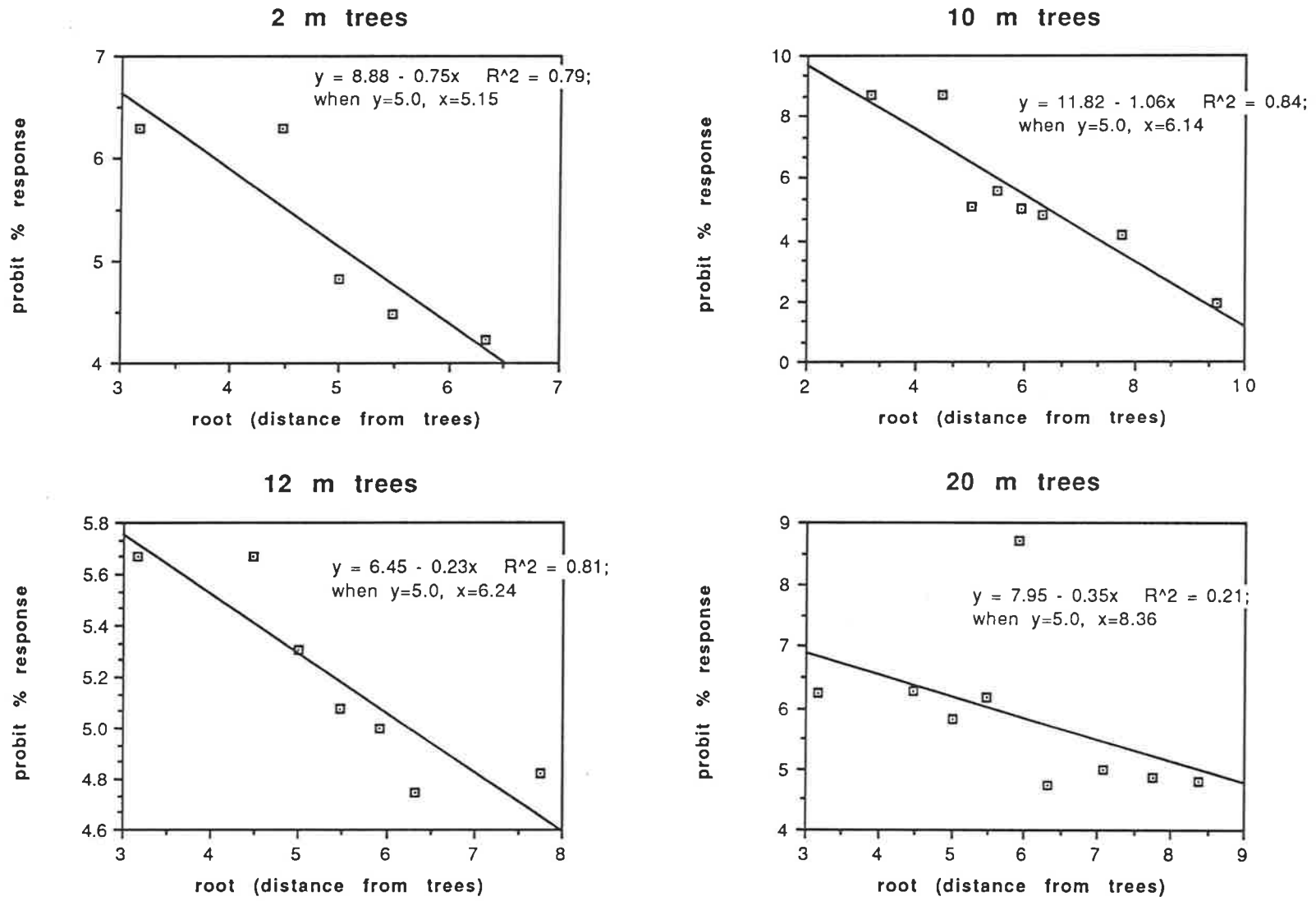


Figure 5.2.3. The probit of the percentage of trigonalyids flying to trees in relation to the square root of the distance from the trees, for each height class of trees separately.

(probit 5) of trigonalyids fly to the trees can be estimated for each class of tree (Table 5.4); and that distance -or its square root- can be regressed on height of tree (Figure 5.2.4). The regression of the square root of the distances provides the highest regression value. An outlying data point is evident for the 20m tree (Figure 5.2.2, 5.2.3), and if this "outliner" is omitted, the regression value is still higher ($r>0.96$, $P<0.05$; Table 5.4). This result indicates that as tree height increases, the point at which 50% of trigonalyids fly to the trees moves further from the trees as a function of the square root of the trigonalyid to tree distance.

Similar graphs of the probit of percentage responce against some function of the angle to the top of the trees suggested that the 'best' function of the angle was "90° minus angle to canopy". The appropriate graphs are presented in Figure 5.2.5. From these graphs, the angle at which 50% (probit 5) of *T. venatoria* fly to the trees can be estimated. Unlike the distances in Figure 5.2.4, these angles all fall within a narrow range of 13° (73.23 to 85.57; Table 5.4), or 15° if the "outlier" is omitted (Table 5.4). The angle for the 10m non-host tree also falls within this range (73.23°). The results suggest that the apparent height of the trees on the visual horizon of *T. venatoria* has a significant effect on the percentage of insects that fly to the trees.

The relatively small sample sizes and large variation in responses do not allow further analysis of the data. However, (1) a significant regression of percentage response on distance was obtained, despite the data being recorded under a variety of wind conditions; (2) a critical distance was evident at which the response of *T. venatoria* dropped suddenly, and this distance increased with the height of the tree; (3) the height of the trees on the visual horizon of *T. venatoria* consistently affected the insect's response to the trees; and (4) the response of the *T. venatoria* to the 10m non-host elm trees was similar to their response to the eucalypts. The combined evidence points overwhelmingly to the conclusion that, in the field, trigonalyids use visual rather than olfactory cues to locate the habitat of their hosts.

T 5.4

The distance, square root of the distance and angle at which 50% (probit 5) of trigonalyids fly to the trees in each height category. The distances have been calculated from the regressions in figures 5.2.2, 5.2.3 and 5.2.5, both with and without the "outlier" point in the 20m category. The regression value and probability are given for the regression of each set of distances on the height of the trees.

Height of tree (m)	Distance at probit 5	Dist. at probit 5 (- outlier)*	√Distance at probit 5	√Dist. at probit 5 (- outlier)*	[90°-angle] at probit 5	[90°-a] at prob 5 (- outlier)*
2	27.74	27.74	5.18	5.18	85.57	85.57
10	45.01	45.01	6.14	6.14	73.23	73.23
12	40.51	40.51	6.24	6.24	73.86	73.86
20	64.17	54.90	8.36	7.37	84.37	70.43

Regression coefficient and probabiliy for regression on height of tree (see Fig. 5.2.4):

r	0.966	0.960	0.965	0.996	0.070	0.913
p	0.0344	0.0402	0.0347	0.0036	0.9302	0.0874

*Ignoring the "outlier" point for the 20m tree in Figs. 5.2.2, 5.2.3 and 5.2.5.

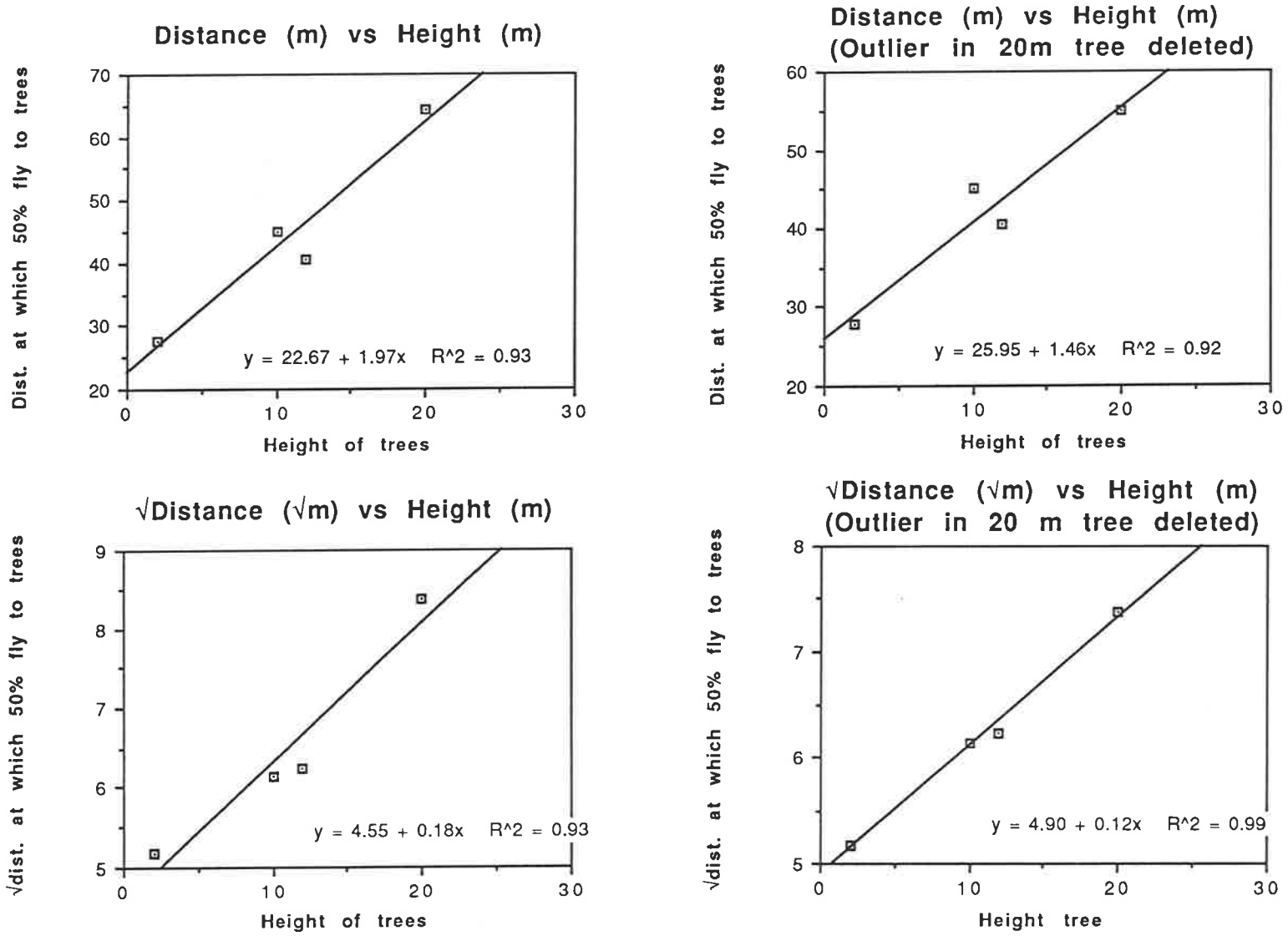


Figure 5.2.4. The distances, and square root of the distances, at which 50% of trigonalyids (probit 5) flew to the trees in relation to the height of the trees. Each graph has been plotted from the values in Table 5.4, both including and excluding the "outlier" in the 20m tree.

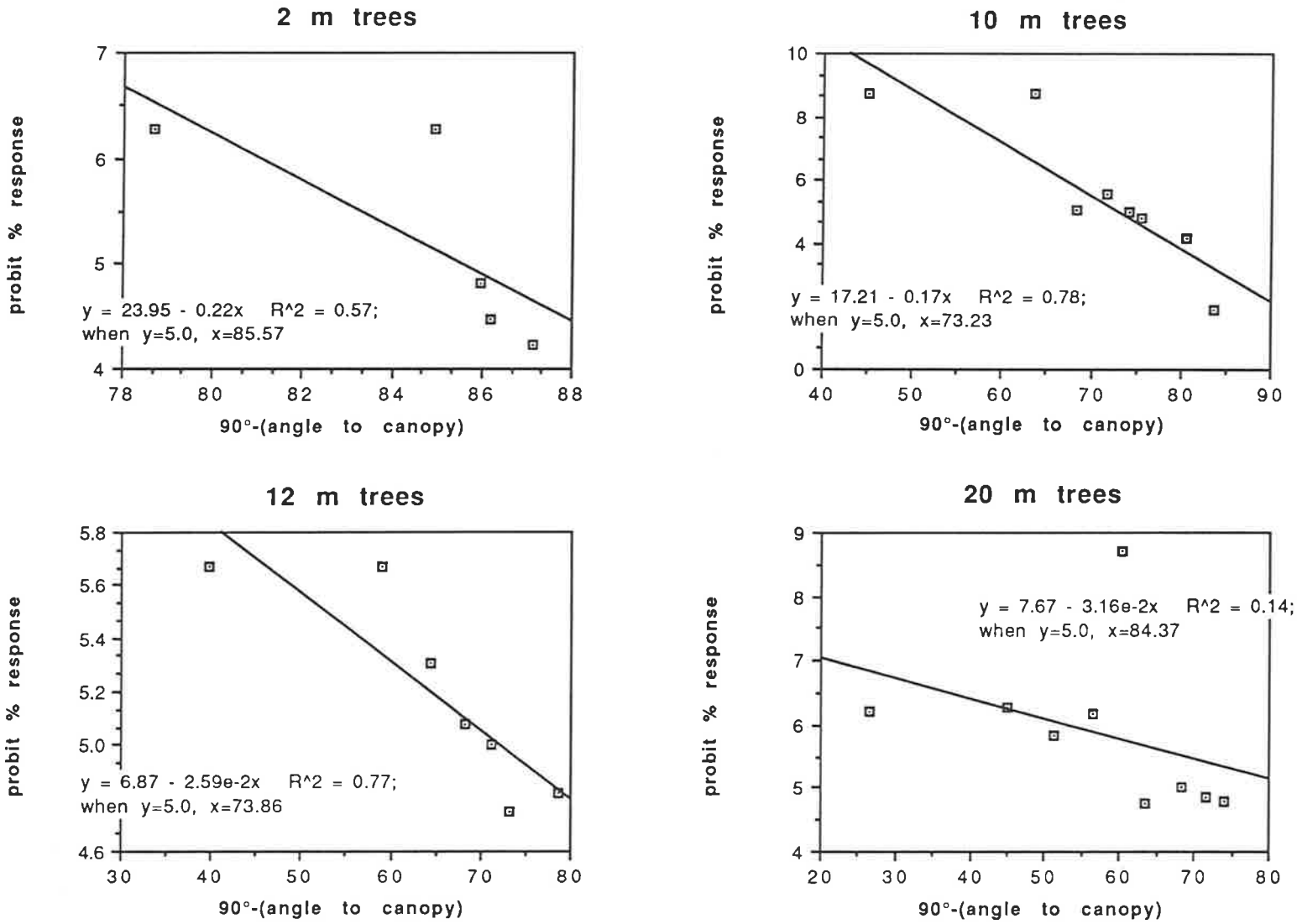


Figure 5.2.5. The probit of the percentage of trigonalyids flying to trees in relation to [90° minus angle to canopy], for each height class of tree separately.

5.5 Section 3. Location and acceptance of oviposition substrate

5.5.1 Materials and Methods

5.5.1.1 Laboratory Studies: Residency times

The residency times of naïve fed and experienced trigonalyids were recorded on host and non-host leaves. A small piece of plasticine was used to attach a young *E. camaludlensis* leaf by the petiole to the centre of the lid of a clear perspex cylinder (15cm diameter x 30cm high). A trigonalyid was then released into the cylinder, and its movements were videorecorded for 15 min. The total time spent in contact with the leaf was calculated by reviewing the recording. The movements of both naïve fed and experienced trigonalyids were recorded, with 4 replicates of each, and using a fresh leaf for each replicate. The procedure was then repeated using young vine-leaves, which were of similar size to the *Eucalyptus* leaves.

The recordings were made in a constant temperature room as described for the olfactometer experiments (5.3.1), with identical lighting, and with the video-camera suspended centrally over the cylinder. The influence on trigonalyid residency time of trigonalyid type and leaf type was analysed by 2 factor ANOVA.

5.5.1.2 Laboratory Studies: Oviposition rates

The oviposition rates of naïve fed and experienced trigonalyids were recorded on a variety of native and exotic plants, and on clean and damaged *E. camaldulensis* leaves. The base of a small branch bearing foliage for oviposition was placed in a 1 litre conical flask, which was plugged with cotton wool. The foliage was then placed in an oviposition cage. Oviposition cages were 1 x 1 x 1m, had mosquito netting walls with access sleeves, transparent plastic fronts through which trigonalyids could be viewed, and contained several honey and water sources (soaked cotton wool). The cages were housed in an outdoor net house at the Waite Institute, and were therefore subject to near natural light, temperatures and wind. They were also exposed to volatiles from surrounding eucalypts and crop plants, as are trigonalyids in the field.

Between 2 and 6 trigonalyids were released into each cage, and kept there for between 5 and 30 'oviposition hours'. Incidental observations had indicated that *T. venatoria* would oviposit only in bright daylight with temperatures above about 15°C. Therefore, records were made only during hours which were suitable for oviposition, for example 8am to 6pm, on a warm and sunny day. The number of 'trigonalyid hours' to which each branch had been exposed was calculated as the product of the number of trigonalyids in the cage by the number of 'oviposition hours'. No interaction was apparent between trigonalyids, and accidental encounters were rare because of the large size of the cages and the ample foliage provided.

Naïve fed or experienced trigonalyids were released into cages containing one of 8 types of foliage, each with at least 3 replicates (= cages), as set out in Appendix 4.5.2. The 8 types of foliage were selected to include plants native to southern Australia (both host plants and non-host plant of *P. dorsalis*), exotic plants, and plants with a variety of leaf physiognomies. A leaf was described as waxy if gently scraping its surface with a scalpel blade (long axis parallel to direction of travel) resulted in the removal of surface wax without damaging epidermal cells; hairy if visibly covered with a dense mat of surface hairs; and hard if it could not be bent through 180° without breaking (loss of ability to return to original shape). The plants and their characteristics are listed in Table 5.5.2. At the end of each trial, the branch was returned to the laboratory and stripped of all foliage. The total length of leaf margin was measured with a hand-held map odometer. The number of trigonalyid eggs that had been deposited along the leaf margins was then counted with a dissecting microscope, and the oviposition rate was calculated as follows:

Oviposition rate = No. of eggs/m of leaf margin/trigonalyid hour.

For the *Eucalyptus* foliage, the leaves were further subdivided into young clean leaves (1.5m tree), older clean leaves (5m tree) or damaged leaves, in order to obtain separate oviposition rates for each type. The effect on oviposition rate of trigonalyid type and foliage type was analysed by 2 factor ANOVA for those species on which oviposition occurred.

5.5.1.3 Field studies

The residency time of trigonalyids released onto foliage in the field was recorded, as was the occurrence or non-occurrence of ovipostion.

The intention was to expose three types of trigonalyids (naïve fed, experienced on *E. camaldulensis* and experienced on *Acacia piquata*) to each of three species of leaf, thereby enabling a ANOVA to be performed to determine the effect on residency time of trigonalyid type and leaf type. Three different types of foliage were selected as substrates; young (2m) *E. camaludlensis* trees, *Vitis vinifera* vines, and young (2m) *Acacia piquata* trees (for characteristics of foliage, see Table 5.5.2). The plants were all within the grounds of the Waite Institute; they were in small clumps of 6-8 individuals, and the vines were in the second row of a small shiraz vineyard. The rims of uncapped holding vials were juxtaposed to leaves, and a 10 min timing interval was started the moment a trigonalyid had climbed onto a leaf. Whilst upon the leaf, or during short 'jump' flights to and upon adjacent foliage, trigonalyids were counted as residing upon the foliage. Timing was stopped if the trigonalyid left the clump altogether, or after 10 continuous minutes of residence. The trigonalyids used were either naïve fed or experienced on *E. camaldulensis*, as in previous experiments. In addition, trigonalyids which were 'experienced' on foliage other than that of *E. camalgulensis* were tested on *E. camaldulensis*; these trigonalyids had been kept for 1-2 days in oviposition cages (as above) containing *Acacia piquata.* Unfortunately, insufficient experienced (*A.piquata*) *T. venatoria* were available, and the exposure of experienced (*A.piquata*) *T. venatoria* to the leaves of *A. piquata* and *V. vinifera* was omitted. All other treatments were completed, as set out in Table 5.7.1.

The hypothesis that residency time was affected by trigonalyid type and leaf type was tested by one factor ANOVA, and, where possible, by 2 factor ANOVA. The hypothesis that occurrence or non-occurrence of oviposition was affected by trigonalyid type and leaf type was analysed by contingency table analysis.

In order to compare the oviposition substrates accepted by *T. venatoria* to those recorded for the other trigonalyids, a list of known

trigonalyid oviposition substrates was compiled from the literature. This list is presented as Table 5.8.

5.5.2 Results

5.5.2.1 Laboratory studies: Residency times

The trigonalyid type, leaf type and residency times and variances are given for each treatment in Table 5.5.1 together with the mean percentage residency time for each treatment.

It is clear from Table 5.5.1 that the variance in treatment 2 (experienced trigonalyids on *E. camaldulensis*) is much larger than that in the other treatments, with two of the four residency times in treatment 2 being longer than the total residency time in other treatments. Heterogeneity of variances was confirmed for the treatments by Bartlett's test (Zar 1984) (X^2=10.33, df=3, P<0.05). To normalise the variances for the 4 treatments, the residency time for each replicate was transformed to natural logs (Table 5.5.1). The transformed data were then used to test the hypothesis that residency time is not affected by trigonalyid type or leaf type.

The analysis, by ANOVA, is given in Appendix 4.5.1. Residency time was not affected by trigonalyid type (P=0.50) or leaf type (P=0.40), but the interaction of the two was significant (P=0.04). Experienced trigonalyids on *Eucalyptus* have a higher mean percent residency time than do trigonalyids in other treatments (Table 5.5.1). The results provide some indication that *T. venatoria* may spend more time on leaves of species on which they have previously oviposition experience. The residency times of *T. venatoria* with oviposition experience on leaves other than *Eucalyptus* are discussed in section 5.5.2.3.

The large variance in residency times of experienced trigonalyids on *E. camaldulensis* (Table 5.5.1) can possibly be explained by the broad criteria used to define 'experience'. It is possible, for example, that a *T. venatoria* with 1 day of oviposition experience on *E. camaldulensis* will behave quite differently to a *T. venatoria* with 2 days of oviposition experience on *E. camaldulensis*.

Table 5.5.1

The residency time of trigonalyid adults on foliage in the laboratory: the trigonalyid type, leaf type, residency time and ln(residency time) are given for each replicate. The variance of residency time and the mean percent residency time are given for each treatment.

Trig type	Leaf type	Replicate	Res time (minutes)	Treatment variance	Mean % res time	ln(res time)
Exp'ed on Eucalyptus	V. vinifera	1	2.20			0.7885
		2	0.30			-1.2040
		3	0.14			-1.9661
		4	0.50	0.912	5.23	-0.6931
	E. camald-ulensis	1	8.62			2.1541
		2	0.60			-0.5108
		3	1.12			0.1133
		4	3.46	13.427	23.00	1.2413
Inexper'ed fed	V. vinifera	1	0.86			-0.1508
		2	0.62			-0.4780
		3	2.22			0.7975
		4	0.82	0.539	7.53	-0.1985
	E. camald-ulensis	1	0.27			-1.3093
		2	0.28			-1.2730
		3	0.68			-0.3857
		4	1.29	0.230	4.20	0.2549

Table 5.5.2

The species and special leaf characteristics of the 8 types of foliage upon which oviposition rates were recorded.

Plant species	Origin	Host status	Leaves
Eucalyptus camaldulensis	Native to S.A.	Known host	Waxy
Acacia iteaphylla	Native to S.A.	Non - host	
Acacia piquata	Native to S.A.	Non - host	
Banksia marginata	Native to S.A.	Non - host	Hard
Correa reflexa	Native to S.A.	Non - host	Hairy
Hymenosporum flavum	Exotic	Non - host	
Vitis vinifera	Exotic	Non - host	
Cotoneaster sp.	Exotic	Non - host	

5.5.2.2 Laboratory studies: Oviposition rates

T. venatoria did not oviposit on *Banksia marginata* or *Correa reflexa*, presumably because (1) the leaves of *B. marginata* were too hard for the female to penetrate with her ovipositor, and (2) the hairs on the leaves of *C. reflexa* prevented the 'caliper' mechanism of the gaster (see section 3.2.1) from gripping the leaf margins. These species were omitted from the ensuing analysis, leaving 12 treatments for which oviposition rates were compared.

The oviposition rate for each replicate is given in Appendix 4.5.2(a) for the foliage of the 6 different plant species, and in Appendix 4.5.2(b) for the different ages and types of leaf *E. cameldulensis.* The data for all the *E. camaldulensis* "treatments" are pooled in Table 5.6.1, which records the mean oviposition rate for each treatment, and in Table 5.6.2 which records the mean oviposition rate for each trigonalyid type and each species of plant foliage. For *E. camaldulensis* only, the mean oviposition rate on each type of foliage is given in Table 5.6.3.

It can be seen from Table 5.6.1 that the variances of the treatments differed greatly, and heterogeneity of variances was confirmed by Bartlett's test (X^2 =65.67, df=11, P<0.001). To normalise the variances for the 12 treatments, the oviposition rates were transformed to natural logs (Appendix 4.5.2) and the means given are therefore the backtransformed means of ln(oviposition rate). The transformed data were then used to test the hypothesis that oviposition rates are not affected by trigonalyid type or leaf species. The ANOVA (Appendix 4.5.3) indicates that oviposition rate is significantly affected by trigonalyid type (P<0.001), leaf species (P<0.01) and the interaction of the two (P<0.01).

The backtransformed means of ln(oviposition rate) in Table 5.6.2 suggest that (1) naïve fed *T. venatoria* have a greater oviposition rate than do experienced *T. venatoria*, and (2) *Vitis vinifera* , *Cotoneaster* sp. and *Hymenosperum flavum* were oviposited on at a higher rate than were the remaining species. The significance of the differences between individual treatments means was further tested by Fisher PLSD, and the treatment means which did not differ significantly are

Table 5.6.1

Oviposition rates of trigonalyids on various types of foliage, ranked in decreasing order of ln(oviposition rate).
The mean oviposition rate (eggs per metre per hour) and variance is given for each treatment, as well as the backtransformed mean ln(oviposition rate) [Exponential mean ln]. The means which did not differ significantly by Fisher PLSD (at 95% level) are indicated by bars.

Rank mean ln(ovip'n rate)	Leaf species	Trigonalyid type	n	Mean ovip'n rate	Variance	Mean ln(ovip'n)	Exponential mean ln	Significance bars
1	*H.flavum*	Naïve fed	3	21.83	18.40	3.07	21.54	
2	*Cotoneaster*	Naïve fed	3	7.09	17.14	1.86	6.42	
3	*Cotoneaster*	Experienced	3	4.85	1.73	1.55	4.71	
4	*V.vinifera*	Naïve fed	6	5.68	12.36	1.53	4.62	
5	*A.iteaphylla*	Naïve fed	3	2.23	0.18	0.79	2.20	
6	*V.vinifera*	Experienced	6	2.06	0.69	0.65	1.92	
7	*A.piquata*	Naïve fed	6	3.51	15.68	0.57	1.77	
8	*E.camal'ensis*	Experienced	9	1.09	0.40	-0.10	0.91	
9	*H.flavum*	Experienced	6	0.95	0.70	-0.33	0.72	
10	*A.iteaphylla*	Experienced	3	0.64	0.20	-0.60	0.55	
11	*E.camal'ensis*	Naïve fed	15	1.68	7.67	-0.72	0.49	
12	*A.piquata*	Experienced	3	0.20	0.09	-3.10	0.05	

Table 5.6.2

The backtransformed means of ln(oviposition rate) for each type of trigonalyid and each leaf species.

Leaf species	Mean ovip'n rate
E.camal'ensis	0.61
A.iteaphylla	1.10
A.piquata	0.37
V.vinifera	2.97
Cotoneaster	5.50
H.flavum	2.23

Trigonalyid type	Mean ovip'n rate
Naïve fed	1.68
Experienced	0.84

Table 5.6.3

The backtransformed means of ln(oviposition rate) on 3 types of E. camaldulensis foliage for both naïve fed and experienced trigonalyids.

Trigonalyid type	Oviposition rate on E. camaldulensis foliage			Total
	Young clean	Old clean	Damaged	
Naïve fed	1.15	1.46	1.07	1.28
Experienced	1.10	0.81	0.39	0.60
Total	1.12	1.09	0.57	0.85

indicated by signifance bars in Table 5.6.1. Naïve fed *T. venatoria* on *H. flavum* and *A. piquata* had higher oviposition rates than did experienced *T. venatoria* on the same plants. For the remaining plant species, no experienced *T. venatoria* had a higher oviposition rate than did naïve fed *T. venatoria* on the same plants. On *E. camaldulensis* there was no significant difference between the oviposition rate of naïve fed and experienced *T. venatoria*. Naïve fed *T. venatoria* on *H. flavum, Cotoneaster sp.* and *V. vinifera* all had higher oviposition rates than most *T. venatoria* (both naïve fed and experienced) on other plants. The results further support the hypothesis that naïve *T. venatoria* have higher oviposition rates than do experienced *T. venatoria*, and that the highest oviposition rates are on exotic species (Tables 5.6.1, 5.6.2).

For *E. camaldulensis* only, the hypothesis was also tested that oviposition rate was not affected by type of trigonalyid or type of *Eucalyptus* leaves (Table 5.6.3). The analysis of the data, by a 2 factor ANOVA, is given in Appendix 4.5.4. The results indicate that the oviposition rate was not influenced by trigonalyid type (P=0.20), leaf condition (P=0.36), or the interaction of the two (P=0.68). Because *T. venatoria* did not oviposit on hard or hairy leaves (see above), one might have expected the oviposition rate on damaged leaves (which are often hard and/or distorted) to be lower than that on clean leaves; the lack of significance may be due to the relatively small numbers of insects used.

5.5.2.3 Field Studies

The residency time and whether or not oviposition occurred are given for each replicate of each treatment (trigonalyid type x leaf type) in Appendix 4.6.1. The mean and variance of each treatment are given in Table 5.7.1. Because residency times for *T. venatoria* experienced on *A. piquata* were available only for *E. camadulensis* (see Materials and methods and Table 5.7.1), the first hypothesis to be tested was that residency time on *E. camaldulensis* was not affected by trigonalyid type. The analysis is given in Appendix 4.6.2. Residency time of *T. venatoria* on *E. camaldulensis* leaves was found not to be affected by trigonalyid type (F=1.99, df=2, P=0.15). By contrast to the suggestion made in 5.5.2.1 that trigonalyids may spend more time on the leaves of species on which they have previous oviposition experience, this

Table 5.7.1

Residency times of 3 types of T. venatoria released in the field on the leaves of 3 different plant species. The mean residency time (min) and variance is given for each treatment, as is the number of individuals released in each treatment (n) and the percentage of these that oviposited.

Trigonalyid type	Leaf species	n	Mean residency time	Variance	% ovipositing
Exp'ed on Acacia	*E. camaldulensis*	17	3.41	12.69	47
	A. piquata	0	•	•	•
	V. vinifera	0	•	•	•
Exp'ed on E.camal'sis	*E. camaldulensis*	15	5.82	12.46	53
	A. piquata	16	3.52	7.31	44
	V. vinifera	7	3.81	12.78	14
Naïve fed	*E. camaldulensis*	23	4.06	13.81	22
	A. piquata	14	4.42	16.82	57
	V. vinifera	10	3.30	11.12	30

result indicates that the previous experience of a trigonalyid does not affect its residency time on the leaves of *E. camaldulensis*. Alternatively, the lack of significance may be due to the small number of insects used, or to the different environment in which the experiment was conducted.

The occurrence or non-occurrence of oviposition is summarised in Table 5.7.2, and the hypothesis that occurrence or non-occurrence of oviposition on *E. camaldulensis* is independent of trigonalyid type was tested with a X^2 analysis. Comparing observed and expected numbers (see Table 5.7.2), the incidence of oviposition by *T. venatoria* on *E. camaldulensis* leaves was found not to be affected by trigonalyid type (X^2=4.66, df=2, P=0.10). The result concurrs with laboratory findings on *E. camaldulensis* (5.5.2.2). The trigonalyids with oviposition experience on *A. piquata* were not included in further analysis of the data.

The next hypothesis tested was that residency time of naïve fed and experienced (*E. camaldulensis*) *T. venatoria* was not affected by trigonalyid type or leaf type. The results of a 2 factor ANOVA (Appendix 4.6.3) indicate that residency time is not affected by trigonalyid type (P=0.57), leaf type (P=0.33) or interaction of the two (P=0.31). By contrast to the suggestion made in 5.5.2.1 that trigonalyids may spend more time on the leaves of species on which they have previous oviposition experience, this result indicates that the previous experience of a trigonalyid does not affect its residency time on the leaves of *E. camaldulensis, A. piquata* and *V. vinifera*.

The hypothesis that trigonalyid type, leaf type, and presence or absence of oviposition are mutually independent was then tested by three-dimensional contingency table analysis (Zar, 1984; Tabachnick and Fidell, 1989). The analysis (Appendix 4.6.4) indicated that these factors were mutually independent (X^2=9.47, df=7, P>0.05). The incidence of oviposition is therefore not affected by trigonalyid type or leaf type in the field. The result does not contradict the laboratory finding that oviposition rates were affected both by the type of trigonalyid and by the type of leaf (5.5.2.2); in the field, oviposition rates were not determined (see 5.7).

Table 5.7.2

The observed and expected numbers of 3 types of trigonalyids ovipositing or not ovipositing on the leaves of E. camaldulensis, to test the hypothesis that oviposition is independent of trigonalyid type.

	OBSERVED		EXPECTED	
Type of trig	Oviposition	No oviposition	Oviposition	No oviposition
Exper'ed on Acacia	8	9	6.49	10.51
Exper'ed on Euc.	8	7	5.73	9.27
Naïve fed	5	18	8.78	14.22

Total Chi-square = 4.661, df = 2, p = 0.0972; Not significant.

Finally, the residency times when oviposition occurred were compared, by an unpaired t-test, to the residency times when oviposition did not occur, for all replicates and treatments combined. As may be expected, *T. venatoria* stayed on leaves longer when it was ovipositing (mean residency 6.16 min) than when it was not ovipositing (mean residency 2.75 min) ($t=5.51$, $df=100$, $P<0.001$).

5.6 Section 4. Incidential Observations

Video recordings were made of adult female *T. venatoria* ovipositing on young *E. camaldulensis* leaves, and these were replayed frame by frame to determine the duration of one oviposition. Incidential observations of trigonalyid oviposition behaviour were also made, during handling, in oviposition cages, and in the field.

Video recordings demonstrated that one individual oviposition (the actual insertion of the egg into the leaf, taken from the time the abdomen first made contact with the leaf margin) took between 0.5 and 1sec, depending on the age (toughness) of the leaf. One entire oviposition manoeuvre (from the time the body was first rotated away from a linear walk to the time linear walking resumed) took between 1.5 and 2 sec.

Observations indicated that apart from demonstrating higher oviposition rates on softer leaves, as already discussed, *T. venatoria* oviposited more readily on (1) leaves which were large enough to maintain a secure foot-hold upon whilst ovipositing, and (2) leaves which were horizontal, allowing *T. venatoria* to walk along the surface between ovipositions. Individual eggs were nevertheless also found on tiny vertical twigs and in leaf petioles. In soft horizontal leaves, trigonalyids would oviposit around the contours regardless of whether the contour consisted of natural leaf margin, feeding damage by folivores, or a mechanically cut edge. There was no apparent difference in antennation, oviposition or other behaviour upon these substrates. In the oviposition cages and during handling, trigonalyids would occasionally oviposit or attempt to oviposit on the margins of paper, wooden and plastic caging material, and mosquito netting. On large flat surfaces, a female would sometimes undertake a series of 'probes' with the gaster, as though searching for a margin.

5.7 Discussion

Although it is always difficult to interpret negative results, the volume of evidence in this study indicates that trigonalyids do not use chemical cues in 'host selection'. It is possible that the sample sizes used in the two different olfactometers were too small, or that the olfactometers and flight-tunnel were too small for use with a relatively large (1cm) parasitoid. It is likely, however, that if trigonalyids do orient to chemical cues, they would have demonstrated a response strong enough to be detected with at least one of the variety of instruments and odour sources used, as other parasitoids that orient to chemical cues have been shown to do (Drost *et al.*, 1986; Vinson, 1985). Furthermore, the field experiment indicated that visual rather than olfactory cues are used by *T. venatoria* to orient to trees (see section 5.4, Host habitat selection). The use of visual cues is not unusual in dipteran parasitoids (Askew, 1971; Vinson, 1985) and phytophagous insects (see Prokopy and Owens, 1983 for review), but has seldom been reported in hymenopteran parasitoids. When visual cues such as colour have been considered to be significant for Hymenoptera, it is mainly as a secondary factor to chemical triggers (Vinson, 1976, 1984, 1985; Wardle, 1990).

Once landed on a potential oviposition substrate, *T. venatoria* still did not appear to use chemical cues in selecting oviposition sites. Eggs are pushed into the mesophyll of any leaf which the trigonalyid's ovipositor can physically penetrate, at a rate which probably depends on how quickly each egg can be inserted (physiognomy of leaf; see below). For leaves on which *T. venatoria* could physically oviposit, the incidence of oviposition was not affected by the trigonalyid type (naïve or experienced) or by the leaf type (see section 5.5.2.3); yet the oviposition rate was found to depend on both the trigonalyid type and the leaf type (see section 5.5.2.2). These results may indicate that *T. venatoria* undertakes 'test' ovipositions unselectively, and that the feedback obtained from such 'tests' influences the oviposition rate. Both in cages and in the field, oviposition occurred readily on exotic plants which are not food plants for any known host of Australian trigonalyids (see Table 5.7.1 and Chapter 4).

In laboratory studies of residency times on *E. camaldulensis* there was weak evidence that trigonalyids spent more time on leaves if they had previous oviposition experience on the same species (see section 5.5.2.1). However, these results could not be replicated in the field (see section 5.5.2.3). If previous oviposition experience does affect the oviposition rate, it is possible that *T. venatoria* has the capacity for facilitated learning to recognise chemicals from the foliage on which previous generations have succeeded in parasitising hosts (Corbet, 1985). Alternatively, the oviposition rate may be affected by stimuli which *T. venatoria* learns to recognise associatively. Associative learning has been shown to influence host selection behaviour in many parasitoids (for example; Lewis and Tumlinson, 1988; Vet and Schoonman, 1988; Vet *et al.*, 1990), but these parasitoids retain their original chemical stimulus-response pattern, and simply alter their acceptance of different chemical stimuli. In *T. venatoria* chemical stimuli appear to be of minor (if any) importance in triggering host selection behaviour.

Because of their small size, it is almost impossible to locate trigonalyid eggs in the field without prior knowledge of where a female may have oviposited. Therefore, plants not forming part of the experiments conducted here could not be accurately assessed for their potential to act as oviposition substrates. Records in the literature nevertheless indicate that trigonalyids are very catholic in their choice of oviposition substrates. Firstly, the family as a whole uses a wide variety of plant groups for ovipostion, including gymnosperms, monocotyledons and dicotyledons (Table 5.8) and, secondly, several individual species employ a wide spectrum of plant families for oviposition (for example, *T. hahnii* and *P. maga*; Table 5.8). Several authors have remarked that oviposition is apparently random (Townes, 1951; Townes, 1956; Oehlke, 1983a), the eggs being placed "in the foliage of a wide variety of plants, and in some instances, in the petals of the blossoms" (Clausen, 1940), or even on the leaf petiole and stem (Clausen, 1929). Trigonalyids do tend to oviposit predominantly on the one species of plant within a given area, but this does not stop the same species from ovipositing on totally different plants in adjacent areas (Clausen, 1940).

Table 5.8.
Plants used as ovipositional substrates by female trigonalyids (those marked * are for ovipositions in the laboratory; all others are from field observations).

Trigonalyid	Plant	Reference
Bareogonalos canadensis	*Pseudotseuga* sp. (Pinaceae)	Carmean (pers. comm.)
Bareogonalos jezoensis	"A variety of plants"	Vecht, 1933
	Albizzia julibrissin (Mimosaceae)	Matsuura&Yamane1984
	Salix sp. (Salicaceae)	Matsuura&Yamane1984
Lycogaster pullata	*Quercus* sp. (Fagacea)	Townes, 1956
"	*Solidago* sp. (Compositae)	Townes, 1956
"	*Ceanothus* sp. (Rhamnaceae)	Townes, 1956
Mimelogonalos spp.	*Eucalyptus* spp. (Myrtaceae)	Riek, 1954
Orthogonalos pulchella	**Liquidambar* sp. (Hamamelidaceae)	Townes, 1956
"	*Viburnum acerifolium* (Caprifoliaceae)	Townes, 1956
"	*Sericocarpus* sp. (Compositae)	Townes, 1956
Poecilogonalos costalis	? decomposing leaf-litter	Gelhaus, 1987
Poecilogonalos maga	**Lespedeza bicolor* (Leguminosae)	Teranishi, 1929
"	*Trifolium* sp. (Leguminosae)	Clausen, 1931
"	*Bambusa* sp. (Gramineae)	Teranishi, 1929
"	*Rubus palmatus* (Rosaceae)	Clausen, 1931
Poecilogonalos thwaitesi	*Camellia sinensis* (Theaceae)	Clausen, 1929
"	*Euphorbia* sp. (Euphorbiaceae)	Clausen, 1929
Taeniogonalos maculata	*Eucalyptus piperita* (Myrtaceae)	Rodd, 1951
Taeniogonalos venatoria	*Eucalyptus melliodora, E. blackelyi, E. camaldulensis* (Myrtaceae)	Carne, 1969
Taeniogonalos spp.	*Eucalyptus* spp. (Myrtaceae)	Riek, 1954
Trigonalis hahnii	Pinaceae (reared from *Diprion similis*)	n.r. (see Appendix 1)
"	Cruciferae	Oehlke, 1983a
"	*Epilobium angustifolium* (Onagraceae)	Haeseler, 1976
"	*Rubus ? fruticosus* (Rosaceae)	Oehlke, 1983a
"	*Rubus* sp. (Rosaceae)	Haeseler, 1976

Some authors have noted the presence of potential host larvae on the same plant species in the vicinity of ovipositing trigonalyids, and have thought that their presence is an important factor in the selection of the oviposition site (Clausen, 1931; Rodd, 1951). In *T. venatoria* the presence of *P. dorsalis* larvae did not affect host location behaviour (see section 5.3.2). Clausen also observed that oviposition on foliage was "apparently without any relation to the host itself" (Clausen, 1929), and further states that "caged females oviposit quite as readily upon clean foliage as upon that on which caterpillars are present or on which they had previously fed" (Clausen 1940).

In contrast it is clear that the physical properties of a leaf influence trigonalyid oviposition behaviour: *T. venatoria* could not oviposit on hard or hairy leaves, and oviposition rates on the waxy leaves of the known host tree *E. camaldulensis* were often lower than those on non-host trees which did not have waxy leaves (see section 5.5.2.2). Clausen (1929) suggests that a serrate leaf margin may be favoured by some species, and the oviposition rate on the serrate leaves of *V. vinifera* in this study was infact higher than that on several native plant species with smooth, round leaf margins. However, the oviposition rate on other species with smooth round-margined leaves did not differ from the oviposition rate on *V. vinifera*, and it is clear that the effect of leaf shape, colour and surface texture on trigonalyid oviposition rates requires further investigation.

When the oviposition rates for naïve fed and experienced trigonalyids were compared, naïve fed trigonalyids oviposited more readily on a number of leaf species (see section 5.5.2.2). It is possible that the greater oviposition rates seen in naïve *T. venatoria* are the result of these individuals not having experienced a 'correct' eucalypt substrate (learning). However, because naïve *T. venatoria* oviposit at the same rate as do experienced *T. venatoria* on *E. camaldulensis*, (Table 5.6.1), it is more likely that the naïve *T. venatoria* are simply under greater 'oviposition pressure'. The naïve *T.venatoria* would have had up to 10,000 eggs still to be laid (see section 3.3.4), whereas experienced trigonalyids had already been ovipositing for 1-2 days and may therefore have already laid several thousand eggs. The difference in oviposition rates was most marked on the leaves of species on which

oviposition rates were high (*H. flavum*, *Cotoneaster* sp.; Table 5.6.1), and this difference can be explained in simple physical terms; if the leaves are soft and easy to walk upon, oviposition can occur more rapidly; on such leaves, individuals under greater 'oviposition pressure' can increase their oviposition rate, thereby increasing the difference between their own oviposition rate and that of experienced trigoanlyids. This example illustrates why great care is necessary in interpreting a measured variable such as oviposition rate in terms of 'preference'.

In summary, the general pattern of host selection behaviour in *T. venatoria* appears to be one of visual orientation to large objects on the visual horizon (trees), followed by oviposition into any substrate (leaves) whose physiognomy does not prohibit it . *T. venatoria* does not appear to conform to Vinson's (1976) model of successful parasitisation, and may be better compared to phytophagous insects searching for a substrate (see Prokopy and Owens, 1983; Bell and Cardé, 1984; Visser, 1986 for reviews). However, if the 'host selection' behaviour pattern of *T. venatoria* is applied at random locations in southern Australia, a female *T. venatoria* would, in fact, probably oviposit in *Eucalyptus* trees more often than not. Given the diversity of *Eucalyptus*-defoliating insects which can act as hosts (Chapter 4), and the ability of *T. venatoria* to act as both a primary and secondary parasitoid (see section 4.3), the selection pressure to evolve a more precise host selection strategy may not have existed. Other species of trigonalyids also appear to exploit hosts which are to be found on the dominant vegetation type in an area; for example, trigonalyids in Japan and Canada oviposit on bamboo and fir-trees respectively (Table 5.8). Some phytophagous insects which exploit dominant eucalypt vegetation are equally unselective about their oviposition substrate. Hepialid moths, for example, fly low level 'runs' over woodlands, scattering tens of thousands of eggs randomly (McQuillan and Forest, 1985; New, 1989); their emergent larvae are, admittedly, more mobile than those of trigonalyids.

Compared to other parasitoids, which have an egg number at least 1-2 orders of magnitude smaller than that of trigonalyids (Askew 1971), and which follow Vinson's model of host selection more precisely,

trigonalyids must surely represent the extreme of r-selection in parasitoids.

The evolutionary implications of trigonalyid host selection strategy are discussed in relation to the behaviour of *P. dorsalis* in the General Discussion.

CHAPTER 6

THE INFLUENCE OF TEMPERATURE, SOIL MOISTURE AND LARVAL DRY WEIGHT ON PROLONGED DIAPAUSE IN A HOST- PARASITOID COMPLEX.

CHAPTER 6

The influence of temperature, soil moisture, and larval dry weight on prolonged diapause in a host-parasitoid complex.

6.1 Introduction

The synchronisation of host and parasitoid lifecycles is a prerequisite for the continuing existence of the parasitoid, and many parasitoids achieve synchronisation by regulating the physiology and development of their hosts (Vinson, 1975; Vinson and Iwantsch, 1980). In the parasitic wasp *Taeniogonalos venatoria* Riek (Hymenoptera; Trigonalyidae), no such host regulation is apparent, and the completion of development is dependent on the pupation of its host, *Perga dorsalis* Leach (Hymenoptera; Pergidae) (see section 3.4.3). Eonymphs of *P. dorsalis* (the final non-feeding instar) diapause in cocoons in the soil during the South Australian summer, and trigonalyids, when present, diapause as first instar larvae within them (see 3.4.3). Both sawflies and trigonalyids may emerge the following autumn, or may undergo prolonged diapause (*sensu* Hanski, 1988) for up to 4 further seasons (Chapter 3; Carne, 1969), still emerging in synchrony with later generations. The stimuli which lead diapausing *P. dorsalis* eonymphs to pupate are therefore likely to ultimately be those which also, through the resultant changes in host physiology, lead to the emergence of adult trigonalyids. It was the aim of this study to determine what those stimuli are likely to be.

The literature on breaking or prologing diapause in insects is extensive (see Masaki, 1980 and Danks, 1987 for reviews), and many factors have either been shown or been suggested to be of significance. In the Symphyta alone, these factors include

photoperiod and light quality, temperature, water availability, population density, quality and quantity of available foliage, and depth of location of cocoons underground (Carne, 1969; Price and Tripp, 1972; Geri *et al,* 1988; Geri and Goussard, 1988a, 1988b, 1989). From this array of possible stimuli, an attempt was made to select those which were likely to be of most significance in an insect which diapauses some 40cm underground, during the summer, in southern Australia. The likely importance of temperature was demonstrated by Carne (1969), who showed that the incidence of prolonged diapause in another pergid sawfly, *Perga affinis* Kirby, was higher after exposure of the eonymphs to high temperatures. He also observed that a high proportion of *P. affinis* eonymphs remained in diapause following hot dry summers in the field, but he did not investigate water availability, which has been shown to be significant for other insect species diapausing over summer. For example, many South Australian autumn flying moths emerge from underground cocoons after soaking rains (McQuillan and Forrest, 1985); and in *Aphodius tasmaniae* Hope (Coloeoptera; Scarabaeidae), which also diapauses in the soil in southern Australia, larval water loss is significant in delaying pupation (Maelzer, 1961). It is therefore possible that the desiccation of eonymphs of *P. affinis* rather than their exposure to high temperatures *per se* resulted in the increased incidence of prolonged diapause observed by Carne (ibid). The following study was carried out to clarify the role of both temperature and moisture availability in breaking diapause* in *P. dorsalis* larvae, some of which contained trigonalyid parasitoids.

* 'Breaking diapause' is used here in the broadest sense. It may be, for example, that water availability influences emergence only after the completion of diapause development (Andrewartha, 1952; Danks, 1987). However, we are interested here only in differentiating individuals emerging in the same season from those emerging one or more seasons later.

6.2 Materials and Methods

Insects. From the 20th to 23rd November 1988, cocoon masses of *P. dorsalis* were dug up from under a *Eucalyptus camaldulensis* tree at the Waite Institute, where sawflies were known to have buried themselves. The larvae had all been collected in Adelaide as first instars during the 3rd week of May 1988, and were reared together on this same tree until soil entry. The masses were separated into 1512 individual cocoons, which were randomly distributed into 24 groups of 63 cocoons. Each group was placed about 5cm from the bottom of a 30cm diameter plastic pot, which was filled with moist soil (U.C.L.A. formula) to within 2cm of the brim.

Treatments. The pots were allocated randomly to 8 different treatments of 4 temperatures [30°C, 25°C, 18°C, and Field (= mean of 24°C)] x 2 levels of soil moisture (wet or dry), with 3 replicates of each. Constant temperature (±1°C) rooms were used to maintain the pots at the first 3 temperatures, and 16L:8D (standard flourescent lighting). The pots for the field treatments were buried in the shade of a *E. camaldulensis* tree at the Waite Institute, with the pot rims level with the surrounding soil. The cocoons were thus approximately 25cm below the surface, where the average soil temperature was 24°C from December to early February (Waite Institute meterological data).

In the dry treatments at each temperature, each pot was simply allowed to dry out; for that in the field, a convex tarpaulin was placed 50cm above the pots to keep out rain, and an earthen mound uphill from the pots ensured that no run-off flowed into them. In the wet treatments, each pot was stood in 5cm deep water for 24 h at monthly intervals, allowing the soil to become soaked. For the wet field treatment, the pots were left exposed to natural rainfall.

These treatments were maintained for 20 weeks, and covered the normal emergence season of *P. dorsalis* and *T. venatoria* (March and early April).

Data. The following data were recorded :

(1) Water content of soil. The soil percent water content was determined by measuring wet and dry weights of 60g (approx.) soil samples taken from each pot. Samples were taken from about 10 cm below the soil surface, and each pot was sampled fortnightly without replacement.

(2) Dry weight and percent water content of eonymphs. The initial dry weight and percent water content of eonymphs were determined by measuring (a) the wet weight of individuals immediately following cocoon dissection, and (b) the dry weight of individuals after drying for 7 days at 60°C. Two eonmyphs were retained for these weights from each pot (N=48) at the start of the experiment.

Wet and dry weights were also determined for subsamples of eonymphs (9 ± 3 per pot) once during the course of the experiment (at 85 days) and again at the end (125 days) for the 238 of the 327 eonymphs which had remained in diapause. The 85 day period was chosen because that was about the time at which the first eonymphs were due to pupate in mid-February (see emergence phenology, section 3.4.3). Eighty nine diapausing eonymphs were damaged during cocoon dissection, and were omitted.

(3) Percent emergence of adults and numbers of eonymphs undergoing prolonged diapause. In late February, netting was placed over each pot to trap and record daily emergences of adults. The emergence of either a sawfly or a trigonalyid was taken to indicate that the diapause of the eonymph had been broken. This was possible because trigonalyids develop on pupating hosts only, one per host, and do not appear to regulate the diapause/pupation of their hosts (see emergence phenology 3.4.3). At the end of the experiment, all remaining cocoons were dissected to determine the number with either perished contents or diapausing eonymphs.

The percent emergence of adults for each pot was based only on those cocoons which were either empty or which contained viable eonymphs;

$$\text{Percent emergence} = \frac{\text{Nº. sawflies and trigonalyids emerging X 100}}{\text{Nº. emerging + Nº. in prolonged diapause}}$$

(4) Percent Mortality. Percent mortality was determined for each pot by dissecting the 1256 cocoons which remained in the pots at 125 days. Cocoons containing dessicated eonymphs, viable parasitoids other than trigonalyids, the remains of parasitoids other than trigonalyids, or fungally infected contents, were all counted as having died, and percentage mortality was calculated as:

$$\text{Percent mortality} = \frac{\text{Nº. died} \times 100}{\text{Total Nº. coccons left in pot at 125 days}}$$

Analysis. Two-factor ANOVAS were carried out to determine the effect of the temperature and wet/dry treatments on each of soil percent water content, eonymph dry weight and percent water content, percent emergence, and percent mortality. The water contents of the soil and eonymphs at 85 days were used for relevant calculations because physical differences between larvae destined to emerge or to continue to diapause were likely to be most pronounced in the days immediately preceeding pupation (see 2). Finally, the eonymph dry weights and percent water contents which were measured at 0, 85 and 125 days were used to test the hypotheses that neither eonymph dry weight nor eonypmh percent water content were dependent on time.

6.3 Results

(1) Soil percent water content. The soil percent water content of each soil sample and the times at which they were taken are given in Appendix 5.1.1, and the mean soil percent water contents on day 85 of the experiment are shown for each treatment in Table 6.1.1. A typical soil percent water versus time profile is illustrated in Figure 6.1. for the wet and dry 25°C treatments (replicate 1), and the values on day 85 which were used in ensuing analyses are indicated by arrows.

The analysis of the data (two factor ANOVA) is given in Appendix 5.1.2. The soil percent water content was significantly influenced by the temperature of the soil ($P<0.001$), the wet or dry treatment of the soil ($P<0.01$), and the interaction ($P<0.05$). As may be expected, soil percent water content was lowest in the hot dry treatments, and highest in the cold wet treatments (at T=85 days).

(2) Eonymph dry weight and percent water content. The dry weight and percent water content of each eonymph are given in Appendix 5.2.1. The means for each treatment at 85 days are summarised in Table 6.1.1, where the pre-treatment means are also given.

The analyses of the data (two factor ANOVA) are given in Appendix 5.2.2 a,b. The dry weight of the eonymphs (Appendix 5.2.2a) was significantly influenced by the temperature ($P<0.001$) and the interaction between temperature and wet or dry treatment ($P<0.01$), but there was no difference between wet or dry treatment alone ($P=0.46$). Lower eonymph dry weights were recorded at the higher temperatures.

By contrast, the percent water content of eonymphs (Appendix 5.2.2b) was significantly influenced by wet versus dry treatments ($P<0.001$), as well as by temperature ($P<0.001$) and the interaction of the two ($P<0.001$). As might be expected, the percent water contents of eonymphs in the wet treatments were higher than those of eonymphs in the dry treatments. But surprisingly, the percent water content of eonymphs held at 25°C and 30°C was higher than that of eonymphs held at 18°C or in the field (Fisher PLSD, Appendix 5.2.3, $P<0.05$).

Table 6.1.1.

The mean soil percetage water content, the mean eonymph dry weight (grams), and the mean eonymph percetage water content on day 85 of the experiment, for each treatment. Pre-treatment means are also given in the last row for comparison.

SOIL WATER				EONYMPH PROPERTIES				
Treatment	Replicates	Mean soil % water	Standard deviation	Total no. in treat	Mean dry weight	Standard deviation	Mean % H2O content	Standard deviation
30º Dry	3	0.97	0.15	30	0.26	0.07	67.22	2.83
25º Dry	3	3.97	1.96	38	0.24	0.06	69.85	3.39
18º Dry	3	7.30	2.10	27	0.25	0.06	66.09	2.39
Field Dry	3	3.40	1.68	19	0.27	0.05	63.39	2.89
30º Wet	3	16.80	2.79	18	0.20	0.05	74.12	4.38
25º Wet	3	18.00	2.74	32	0.24	0.07	70.88	3.92
18º Wet	3	20.57	2.57	21	0.26	0.07	66.29	2.72
Field Wet	3	8.87	5.62	23	0.30	0.06	64.91	2.57
Pre-treat	8	18.30	0.18	48	0.24	0.08	69.51	3.30

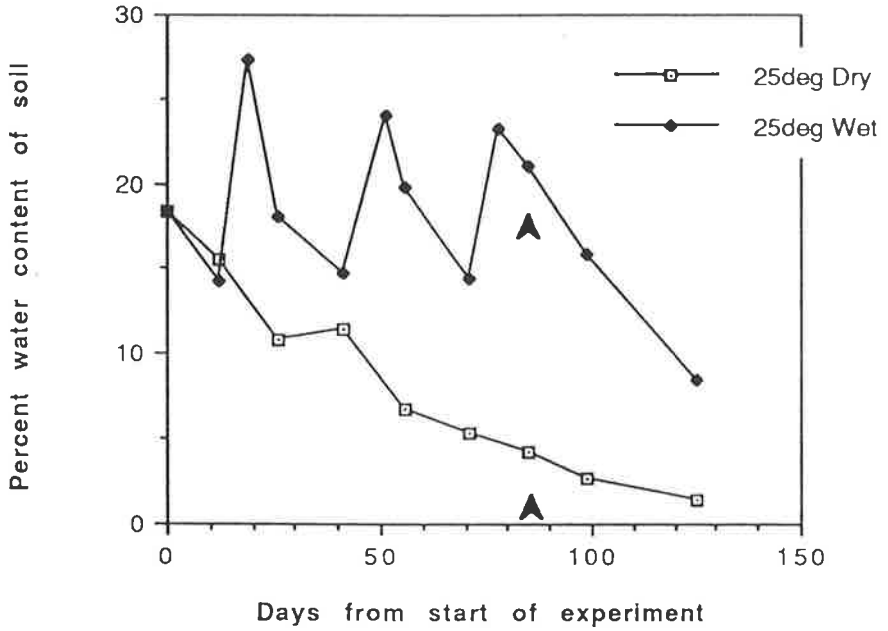


Figure 6.1. The variation of soil percentage water content with time (days from the start of the experiment), for the first replicate of the 25deg Wet and 25deg Dry treatments. The arrows indicate the values at T=85 days, when samples of eonymphs were taken, and the data used in ensuing analyses.

Although the dry weights of some of the eonymphs were influenced by some of the treatments it was of interest to compare the overall mean at each of 0, 85 and 125 days. The overall mean dry weights at 0, 85 and 125 days are given in Table 6.1.2 and the ANOVA is given in Appendix 5.3.1. There was no difference between the means.

It was also of interest to know whether there was a relationship between the percent water content of eoymphs and their dry weight. The data for all 494 eonymphs were therefore plotted (Figure 6.2), and the apparent regression analysed. The regression was significant ($r = -0.41$, $P<0.001$) with a negative regression coefficient, possibly because larger eonymphs have a greater fat content, and hence a relatively lower water content.

Similarly, it was of interest to know whether the treatments caused any overall change in eonymph percent water content during the experiment. The mean eonymph percent water content at 0, 85 and 125 days are given in Table 6.1.2, and the ANOVA and comparison of means are given in Appendix 5.3.2. The mean eonymph percent water content was lower at 125 days than at either 0 or 85 days, which is not surprising.

(3) Percent Emergence. The percent emergence for each replicate is given in Appendix 5.4.1, and the mean percent emergence for each treatment is listed in Table 6.2. Percent emergence was significantly influenced by temperature ($P<0.001$), but not by wet or dry treatment ($P=0.27$) or its interaction with temperature ($P=0.76$) (two factor ANOVA, Appendix 5.4.2a). Percent emergence decreased with increasing temperature (Fisher PLSD, Appendix 5.4.2b, $P<0.05$). It should be noted that although wet treatments had seemingly higher percent emergences than the corresponding dry treatments (Table 6.2) the ANOVA indicated that, overall, the mean difference between wet and dry treatments was not significant. This lack of significance may have been due to the considerable variation in percent emergence within the 30°C and field treatments (see Appendix 5.4.1).

In an attempt to gain further insight into the significance or otherwise of the wet versus dry treatment, the soil moisture content of each pot at 85 days was regarded as a variable (rather than as a constituent of the wet or dry treatment) and the percent emergence

Table 6.1.2

The mean dry weight and mean percent water content of eonymphs at 0, 85, and 125 days from the start of the experiment. The comparison of means is given in A 6.3.

Time (days)	No. of eonymphs	Mean dry weight (g)	Standard deviation	Mean % water	Standard deviation
0	48	0.238	0.077	69.51%	3.3
85	208	0.252	0.067	68.02%	4.36
125	238	0.261	0.062	62.76%	6.01

Fig. 6.2. Regression of percent water content of eonymphs on dry weight of eonymphs, for the 494 eonymphs sampled during the experiment (at 0, 85 and 125 days); r= -0.41, p < 0.001

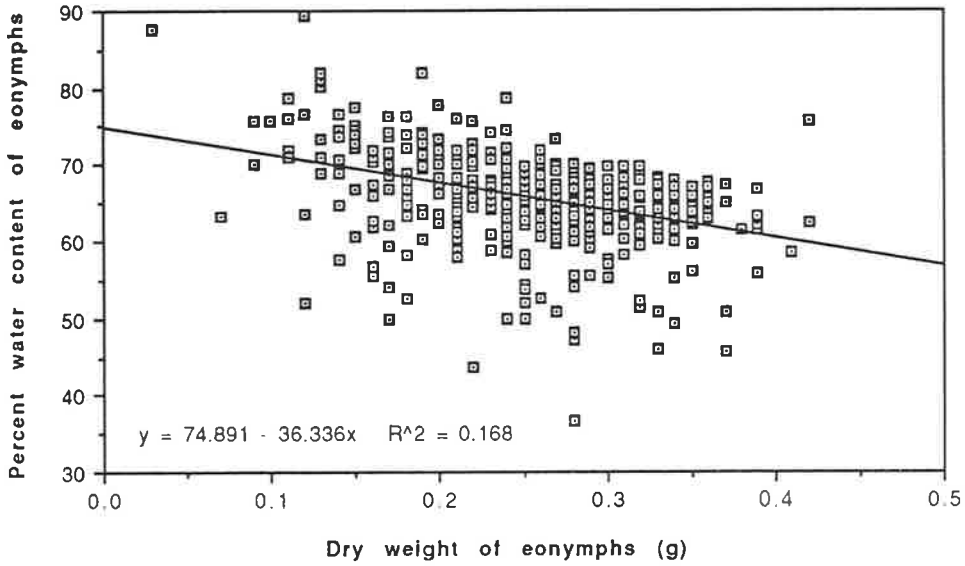


Table 6.2

The average percentage emergence and average percentage mortality for each treatment at the end of the experiment (125 days).

Treatment	N 1*	Mean % emerg per pot	Standard deviation	N 2*	Mean % mort per pot	Standard deviation
30 deg Dry	64	12.1	11.3	153	43.6	27.6
25 deg Dry	101	32.1	4.0	144	36.0	8.5
18 deg Dry	84	63.7	15.3	156	46.3	8.4
Field (24) Dry	49	25.9	22.4	164	68.5	3.7
30 deg Wet	41	14.3	15.3	165	71.6	20.7
25 deg Wet	85	41.7	19.5	152	59.2	14.8
18 deg Wet	43	63.9	16.3	162	71.0	7.6
Field (24) Wet	55	45.3	22.9	160	70.9	17.4

* N 1 = Number of potential emergences (Number emerged + Number in prolonged diapause)
* N 2 = Number of cocoons in replicate at 125 days (after subsampling eonymphs at 0 and 85 days).

from each pot (Appendix 5.4.1, Appendix 5.4.3) was regressed on the soil moisture in that pot at 85 days (Appendix 5.1.1, Appendix 5.4.3). The analysis is given in Appendix 5.4.4. The regression was not significant ($r=0.22$, $df=23$, $P=0.30$).

Similarly, the average eonymph percent water content for each pot at 85 days (Appendix 5.4.3) was then regarded as a variable, and the percent emergence for each pot was regressed on it. The analysis is given in Appendix 5.4.5. The regression was not significant ($r=0.32$, $df=23$, $P=0.12$). Finally the regression of percent emergence for each pot on the average eonymph dry weight for that pot was calculated, but was also not significant (Simple regression, Appendix 5.4.6; $r=0.11$, $df=23$, $P=0.622$). Therefore, it could not be concluded that moisture affected percent emergence in any way, be it through wet or dry treatment, through soil moisture content, or through the percent water content or dry weight of eonymphs.

(4) Percent Mortality. The percent mortality for each replicate (pot) is given in Appendix 5.4.1, and the mean percent mortality for each treatment is listed in Table 6.2. The analysis of the data (ANOVA) is given in Appendix 5.5. The percent mortality was significantly influenced by whether a pot was in a wet or a dry treatment ($P<0.01$), but not by the temperature ($P=0.13$) or by its interaction with the wet/dry treatment ($P=0.50$). Among the laboratory treatments, most deaths occurred in the wet treatments, and it was found upon dissection in week 20 that these deaths were due mainly to fungal infection (*Beauveria* sp., which accounted for 49.8% of total mortality for all wet laboratory treatments combined, and 63.1% of total mortality for the 18°C wet treatment). In the dry laboratory treatments, deaths were due mainly to desiccation (66.8% of total mortality for all dry laboratory treatments combined, and 79.3% of total mortality for the 30°C dry treatment). In the field treatments, many deaths resulted from predation by soil fauna upon the isolated cocoons, which appear far more vulnerable to such predation than those in natural, fused masses.

One might expect mortality to be negatively correlated with the dry weight of the eonymphs, because smaller individuals are theoretically more susceptible to desiccation. In this study, however, there was no significant regression ($r=0.131$, $P=0.54$; Appendix 5.6) of percent

mortality in each replicate (Appendix 5.4.1, Appendix 5.4.3) on the average dry weight of eonymphs in that replicate at 85 days (Appendix 5.4.3). Because the different causes of mortality vary so much between treatments, further attempts at regressing mortality on any of the measured variables would be of dubious value.

6.4 Discussion

In this experiment, an attempt was made to analyse the environmental factors which influence the length of diapause by acting at the eonymph stage of sawfly development. Many factors may also influence the length of diapause by acting on earlier larval stages (Danks 1987, Geri and Goussard 1988a, Zaslaviski and Umarova 1990), but the larvae were all reared under identical conditions so as to minimise those effects. Environmental factors which had potentially exerted their influence by acting on the previous generation or egg stage could not be controlled for. Neither could internal (genetically programmed - *sensu* Danks, 1987) mechanisms be controlled, and it can be argued that the observed differences between eonymphs were the result of preprogrammed differences in diapause behaviour. The ensuing discussion assumes that the converse is true, i.e. that the observed differences between eonymphs were the result of the imposed treatments.

Eonymph water content and dry weight. In nature, the percent water content of the soil surrounding the eonymph in summer is reduced because evaporation is much greater than rainfall. Since eonymph percent water content is expected to be dependent on soil water content, one might have expected to find eonymphs with lower percent water contents in the higher temperature treatments of the experiment. Surprisingly, however, the eonymphs kept at high temperatures had higher percent water contents (Table 6.1.1; Appendix 5.2.2b). Two possible explanations for this result are:

Firstly, that basal metabolic rates are higher at high temperatures, and larvae may metabolise more fat, thereby increasing their percent water content and reducing their dry weights. In this study, dry weights of eonymphs were shown to be reduced at high temperatures (Table 6.1.1, Appendix 5.2.2a), supporting this explanation.

Alternatively, larvae may be physiologically more capable of absorbing water from their surroundings at higer temperatures.

The higher eonymph percent water content at high temperatures indicates that eonymphs were unlikely to have dessicated during the course of the experiment. Nevertheless, eonymph percent water content was found to decrease with time (Appendix 5.3.2), and a possible explanation is that the smaller eonymphs with a high percent water content pupated and emerged earlier than larger eonymphs. Eonymphs which were on average larger, with a lower percent water content, could thereby have been left in diapause, and the percent water content of eonymphs at the end of the experiment was therefore lower than at the start. This explanation would be supported by a positive correlation between eonymph dry weight and time; the results in this study were however only suggestive of such a correlation (Appendix 5.3.1). If eonymph dry weight does increase with time, this is possibly also a result of a higher mortality in smaller eonymphs. No such weight-mortality correlation could be demonstrated in this study (Appendix 5.6).

Although a complex interplay of uncontrolled factors affected the water content and dry weight of eonymphs, the results overall indicate that the percent water content of eonymphs remaining in the soil decreased with time, and that, when possible, such eonymphs absorbed water from their immediate surroundings.

Eonymph emergence and mortality. In many tropical insects water availability and the state of larval hydration are known to directly affect the timing of diapause termination (Okuda, 1988). In this study, it was not possible to correlate the state of hydration of individual larvae at various times with the number of them emerging from diapause, because percent water content of eonymphs had to be determined by destructive subsampling of cocoons. However, the data obtained (Appendix 5.4) indicate that none of the moisture variables examined (wet or dry treatment, soil moisture content, eonymph percent water content) affected the percent emergence from diapause. High temperatures, on the other hand, significantly reduced the percent emergence from diapause (Appendix 5.4.2). Increased water availability may nevertheless have contributed to diapause termination during some critical period of eonymph sensitivity, but temperature clearly had a more significant long term influence on diapause behaviour than did water availability.

Lowered larval weights have been shown to reduce emergence (prolong diapause) in sawflies when the lowered larval weights are the result of competition for resources; thus when densities of sawfly larvae were high, the individuals were smaller, and a larger proportion of the population entered prolonged diapause (Carne, 1969; Geri and Goussard, 1989). Density dependent prolonged diapause has also been demonstrated in other insects (Tauber *et al.,* 1986; Danks, 1987; Hanski, 1988) and larvae in density-dependent prolonged diapause induced by a shortage of resources have smaller fat reserves (and higher percent water contents) than larvae not in prolonged diapause. By contrast, in populations of species which are polymorphic for diapause but which do not undergo density-dependent diapause, diapausing individuals usually have larger fat reserves (Danks, 1987). Both diprionid and pergid sawflies demonstrate density-dependent prolonged diapause, and there is evidence to indicate that 'crashes' in outbreak populations of these pest species result from an increasing proportion of the population undergoing such prolonged diapause (Eichhorn, 1982), possibly in response to changes in the quality of foliage (Kolomiets *et al.,* 1979; Geri, 1988; Myers, 1988). In tenebrionid beetles, pupation is also known to be inhibited by crowding (Nakakita, 1982), and Nakakita (1990) has demonstrated the neuroendocrinological mechanism by which contact with other individuals leads to high juvenile hormone levels and inhibition of pupation.

In this study, high temperatures were shown to reduce both the dry weight of eonymphs (Appendix 5.2.2) and the percent emergence of adults (Appendix 5.4.2). However, the regression of percent emergence on eonymph dry weight was not significant (Appendix 5.4.6), so it is unlikely that lowered larval weight was the mechanism by which high temperatures reduced percent emergence.

Eonymph mortality in wet soil was high due to fungal infection, and in dry soil because of desiccation. An optimal soil moisture regime presumably exists, and is likely to be dependent on local rainfall. Thus *P. affinis* larvae in New South Wales and Victoria occurr predominately in areas with an annual rainfall between 450 and 700mm (18 and 28 inches) (Carne, 1969), and the distribution of *P.*

dorsalis larvae (surveyed in Chapter 2) is also restricted to such a rainfall belt (500 to 1000mm/annum).

Observations in the field indicated that predation upon eonymphs in isolated cocoons was far greater than upon eonymphs in natural cocoon masses. Incidental predators (asilid larvae, coleopteran larvae) are likely to be responsible, because natural cocoon masses are not attacked. Sequestered *Eucalyptus* oils are incorporated into the cocoon during construction (Carne, 1962), and these oils are possibly more repellent to predators when in larger quantities such as found in cocoon masses.

Temporal and spacial distribution of sawflies and their parasitoids. If resource quality/availability (or temperatures) are unfavourable for the survival of actively feeding larvae or ovipositing adults, fitness is presumably optimised by eonymphs remaining in diapause until a subsequent season, despite increasing mortality during diapause. This was demonstrated for *P. affinis* by Carne (1969), and is suggested for *P. dorsalis* by this study. Prolonged diapause is one way of "spreading the risk" of extinction in a variable environment (Den Boer, 1968), and is also employed by other ecological "hedge-betters" in similar habitats, eg. *Antheraea* spp. (Lepidoptera: Saturniidae) (McQuillan and Forrest, 1985; New, 1989). An alternative risk-spreading strategy is that of dispersal and migration, which "spreads the risk" in space rather than in time. The possibility of *P. dorsalis* spreading the risk in space as a complementary strategy to prolonged diapause is discussed in relation to individual behavioural differences between larvae (see Chapter 7).

In conclusion, the findings of the present study support the observation of Carne (1969) that large numbers of sawflies (and trigonalyids) are unlikely to emerge after hot, dry summers. A series of such summers could lead to an accumulation of diapausing eonymphs, and if followed by a cooler, wetter summer, a significant outbreak of sawfly larvae may be triggered.

Further investigation of the factors which influence the spatial and temporal distribution of sawflies and the synchronisation with their parasitoids could lead to the determination of a set of measurable variables for use in assessing the probability of a sawfly outbreak. Of

the factors considered in this study, temperature, by its effect on diapause, was the major determinant of the temporal distribution of adult sawflies and trigonalyids, whereas water availablity, through its effect on mortality, would appear to be the major determinant of their spatial distribution. Temperature can in this contex also be viewed as an important factor in synchronising post-diapause development and emergence, as it has been shown to be in univoltine populations of diprionid sawflies (Hodek and Hodková,1988).

CHAPTER 7

COLONY BEHAVIOUR IN *PERGA DORSALIS*.

CHAPTER 7

Colony behaviour in *P. dorsalis*

7.1 Leadership behaviour in *Perga dorsalis*

7.1.1 Introduction

Sawflies in the genus *Perga* (s.l.) (Hymenoptera: Pergidae) are well known for their gregarious larvae, which are often found resting as cylindrical masses around the branches of *Eucalyptus* trees. The individuals in the colonies disperse to feed upon foliage by night and reaggregate before dawn. To reassemble, larvae communicate by means of low frequency vibrations, created by tapping the uropod upon the substrate (Carne, 1962). This gregarious behaviour is thought to advantage the larvae defensively (Carne, 1962; Jolivet *et al.,* 1991), and to enable them to more easily penetrate soil prior to cocoon formation than could individual larvae on their own (Carne, 1966).

Carne (1962) first suggested the possibility of individual behavioural differences among pergid larvae, recalling the work of Wellington (1957, 1977), who showed that colonies of the western tent caterpillar *Malacosoma californicum pluviale* (Dyar) contained a subset of "active type 1" larvae. When compared to other colony members, these larvae were found to be more robust, more active and better oriented in feeding, faster developing, and gave rise to more highly dispersing and more fecund adults. Looking for indications of a similar subgroup of more active individuals in colonies of pergid larvae, Carne (1962) observed that certain individuals were found with "unexpected frequency" among those leading the colonies to and from their diurnal resting sites. He did not, however, go beyond reporting these observations, and the behaviour has therefore not been quantified.

The following study investigates experimentally the presence of a subgroup of more active individuals in colonies of *P. dorsalis* larvae. For ease of reference, such a subgroup of more active individuals is here referred to as 'leaders'.

7.1.2 Materials and Methods

Two experiments were carried out. Experiment 1 involved the recording of larval behaviour with minimal interference to the colonies. Experiment 2 involved the manipulative relocation of larvae within colonies.

7.1.2.1 Experiment 1 : Recording larval behaviour.

(i) Treatments

Three colonies of fifth instar larvae of *P. dorsalis* were collected from *Eucalyptus torquata* in metropolitan Adelaide in September 1989. These colonies were placed on potted 2m *Eucalyptus camaldulensis* in the laboratory and were left for 2- 3 days to allow the insects to resume normal nocturnal feeding following the disturbance. They were then monitored on a nightly basis. Colony A, consisting of 47 individuals, was studied for 15 consecutive nights (10/9/89 to 2/10/89), and colony B, consisting of 30 individuals, for 12 consecutive nights (20/9 /89 to 4/10/89). The third colony, colony C, acted as a control (see below) and was monitored for 10 consecutive nights (3/10/89 to 12/10/89).

(ii) Labelling and Recording

Larvae were labelled each night when they first moved out to feed after dark. An infrared beam was set up across the larvae's path, and an alarm was wired up to ring when this beam was broken. The experimentor was thus alerted, enabling marking and recording to be carried out before the foraging larvae dispersed. Overhead fluorescent lighting was briefly used during marking and recording; the larvae did not disperse during this disturbance, so their relative positions were not affected. Larvae at both the head end and the tail end of the line of dispersing larvae were labelled because an earlier pilot study had suggested that larvae which 'led' on one night were as likely to occur in the last 3 positions as in the first 3 positions on subsequent nights.

For colonies A and B, the first 3 larvae at the head of the colony, and the last 3 larvae at the tail of the colony, were marked with oil paints every night, irrespective of previous markings. Six different colours were applied to the abdominal sclerites in combinations of dots and dashes, so that individuals could be recognised. The markings were used to record each occurrence of an individual larva in either the first 3 or the last 3 positons; larvae which did not occupy these positions received no markings. On nights when 2 larvae jointly occupied the third position, 8 larvae were marked and recorded; the first 4 and the last 4. In all other cases only 6 larvae were recorded.

For Colony C, consisting of 36 larvae, 4 individuals from the centre of the colony were marked. As with the larvae in colonies A and B, they were marked and recorded each night, for 10 nights. This colony acted as a control to see if marking induced leading.

Paint marks were applied with a fine brush by approaching each larva extremely slowly, so as to not elicit its characteristic defensive reaction of regurgitating sequestered *Eucalyptus* oil. This oil is often smeared onto the terminal abdominal sclerites as a larva arches its head and tail over its midsection (see section 7.2); previous markings could thus be obscured.

(iii) Analysis

There are numerous ways of testing the null hypothesis that all larvae in a colony are equally likely to 'lead' in the nightly foraging expeditions. Only 2 methods were used, hereafter called methods 1 and 2. It was initially assumed, for each method, that on any one night a larva was 'leading' if it occurred in any one of the first 3 or last 3 positions. On nights when 2 larvae jointly occupied the third position, the first 4 and last 4 positions were used to define 'leaders'. Before either of these methods was used, the hypothesis was tested that larvae in the first 3 (or 4) and the last 3 (or 4) positions were interchangeable.

Method 1

H1: All individuals in a colony lead with equal frequency.

This method compares the number of observed (marked) and expected larvae in leading positions, with a 'leading position' being defined as one occupied by any of the first 3 (sometimes 4) or the last 3 (sometimes 4) larvae in a colony on any one night.

For colony A, 6 larvae were marked on each of 10 nights, and 8 on each of 5 nights. There was thus a total of (6 x 10) + (8 x 5) = 100 larvae in leading positions over the 15 nights of observation, with a mean of 100/15 = 6.67 larvae per night. Since there were 47 individuals in the colony, the probability of one larva being in a leading position on a given night by chance alone is 6.67/47 = 0.142.

For colony B, with 30 individuals, 6 were marked on 6 nights and 8 were marked on 6 nights, giving a total of 84 larvae in leading positions over 12 nights of observation, with a mean of 7.0 larvae per night, and a probability of one larva being in a leading position on one night of 7/30 = 0.233.

The probabilities of a larva being in a leading position on 0,1,2,...n nights may be calculated by the binomial theorem ,

$$P = C_x^n \, p^x \, (1-p)^{n-x}$$

assuming that the postions of larvae on each night constitute an independent Bernoulli trial. The numbers of observed and expected larvae in leading positions may then be compared with a χ^2 or a G test (Zar, 1984). The analysis was repeated with 'leading positions' defined both as the first 3 (or 4) positions only, and as the last 3 (or 4) positions only.

Some individuals which had been recorded in leading positions and which moulted during the course of the experiment were recorded as different individuals after moulting, because identification by their original markings was no longer possible (several individuals often moulted on the same night). The average number of occurences in leading positions for each larva would therefore tend to be underestimated, biasing the analysis in favour of H(0).

This first method is equivalent to labelling all the larva on the first night, and recording the frequency with which each individual occurs in a leading position on subsequent nights. The marking of some individuals each night is far easier, however, as many larvae which never occur in leading positions do not require individual identification.

For Colony C, the number of nights upon which any labelled larva was expected to occur at least once in any one of 6 leading positions by chance alone is 5.3 (Binomial theorem). This can be compared to the observed number using a G test (Zar, 1984).

Method 2

H2 : a larva recorded as leading on any night, say n(0), is no more likely to lead again on subsequent nights than would be expected by chance alone.

This method compares the number of observed and expected larvae in leading positions during the 5 or 10 nights following their first labelling.

Leading positions were defined as in Method 1, and the occurrence of each larva in one of these positions was recorded during the 5 or 10 nights following the night of its first labelling.

A larva was included in this analysis if it was first recorded in a leading position more than 5 (or 10) nights before the end of the experiment, and if it then did not moult for the next 5 (or 10) nights.

This analysis has the advantage of using only larvae which have been observed for the same number of nights. Larvae which were first recorded in a leading position after 5-7 nights thus had an equal chance of being recorded in a leading position on n subsequent nights as did the larvae first recorded in a leading position on the first few nights.

The observed numbers of larvae were compared to the numbers expected by chance alone, which were again calculated with the bionomial theorem. As in Method 1, the G test was used because of the low numbers of observed and expected larvae; and it was assumed that the positions of larvae on each night constituted an independent Bernoulli trial.

7.1.2.2 Experiment 2 : Manipulative relocation of larvae

(i) Treatments

Two colonies of third instar *P. dorsalis* larvae were collected from *Eucalyptus camaldulensis* near Mt. Gambier, S.A., in November 1989. The colonies were settled on *E. camaldulensis* as in experiment 1. The colonies, X and Y, consisted of 25 individuals each, and both were studied for 6 days (10/11/89 to 16/11/89).

(ii) Labelling and recording

Prior to the experiment, a few larvae at the front and back of each colony were labelled in colonies X and Y, and these larvae were monitored for 3 – 4 days to select the larva from each colony that lead most frequently. The experiment was then started with this larva being removed from the colony at 12:00 ±1.5 h each day and being placed at the centre of the colony regardless of its original position on that day. Its position in the colony was then recorded on the evening following this manipulation (18:00 ±1.5 h) and again the following morning, after the colony's nightly dispersal and reaggregation (9:00 ±0.5 h). As the larvae were aggregated at the time of recording, the front and back positions were taken as those occupied by resting larvae which were furthest from the centre of the colony (head capsules closest to the tip or base of the branch upon which they were resting).

The subject larva was recorded as either leading (front 2 or back 2 positions only) or not leading (all other positions) over the 5 nights. Because the colonies were relatively small, only 4 positions were considered rather than 6 or 8 as in Experiment 1.

(iii) Analysis

H3 : Larvae remain in the positions into which they are placed following manipulation.

The analysis determines whether the high frequency of an individual occurring at the extremities of a colony is a result of genuine behavioural differences, or whether 'leading' is a purely positional effect, resulting from larvae maintaining their fortuitous positions during nightly dispersal and reassembly. If 'leading' were a positional effect, one would expect a larva which was placed in the middle of the colony to maintain this position. Each larva in colonies X and Y that was placed back into the middle of the colony should therefore reappear in leading positions only by chance. The observed and expected numbers of reappearances in leading positions may be compared by a G test (Zar, 1984).

7.1.3 Results

7.1.3.1 Experiment 1: Recording larval behaviour

1. The effect of marking on leadership behaviour; H3

Only one of the 4 labelled larvae from the centre of colony C was recorded in a leading position on only one night out of 10. A comparison with the expected number of occurrences of larvae in leading positions at least once by chance alone (=5.3), gives a value of $G = 7.35$ (1d.f., $P < 0.01$). The result therefore confirms that marking does not induce leadership behaviour.

2. The interchangeability of the first and last positions; colonies A and B

To test the hypotheses that:

H4: a larva in the **first** 3 positions on one night was equally as likely to occur in the last 3 positions as in the first 3 postions on subsequent nights; and

H5: a larva in the **last** 3 positions on one night was equally as likely to occur in the first 3 postions as in the last 3 positions on subsequent nights.

The relevant data from Experiment 1 are given in Table 7.1. A chi-square analysis of the recurrence of larvae in leading positions (H4, H5) was not significant, indicating, as suggested earlier, that 'leaders' occur interchangeably at either end of the colony. In subsequent analyses, therefore, a larva was considered to be in a leading position if it occurred in <u>either</u> the first 3 <u>or</u> in the last 3 positions in the colony on any one night.

3. **H1:** All individuals in a colony lead with equal frequency

The observed and expected numbers of larvae which were in leading positions on 0,1,2,...n nights are given in Table 7.2a for colony A and 7.2b for colony B.

An appropriate statistical comparison may be made at about 95% of the accumulative probability, i.e. for nights 5 – 15 inclusive for colony

Table 7.1: The frequency of positions on subsequent nights of larvae in colonies A and B which were initially recorded in the first 3 positions (H4) and in the last 3 positions (H5).

Hypothesis	Larvae initially recorded in:	No. of larvae	Frequency of positions on subsequent nights: In first 3	In last 3	χ^2 to test Hypothesis
Colony A :					
H4	First 3 positions	10	13	15	0.14; NS*
H5	Last 3 positions	7	23	24	0.04; NS
Colony B :					
H4	First 3 positions	11	19	13	1.13; NS
H5	Last 3 positions	10	8	16	2.67; NS

* NS = not significant

Table 7.2: The probability of a larva being in a leading position by chance, and the observed and expected numbers of larvae in leading positions on 0,1,2,...n nights; (a) Colony A; (b) Colony B.

7.2(a):

Nights (out of 15)	Probability		Number of larvae: Expected (Prob. x 47)		Observed (out of 47)
0	.1008		4.75		22
1	.2499		11.75		8
2	.2892		13.59		1
3	.2071		9.73		5
4	.1027		4.83		2
5	.0374		1.76		3
6	.0103		0.48		1
7	.0022		0.10		0
8	.0004	P=.0503	0.02	EXP=2.36	1
9	≈ 0		≈ 0		3
10	≈ 0		≈ 0		0
11	≈ 0		≈ 0		1
12	≈ 0		≈ 0		0

G test for comparison of observed and expected numbers (bracketed) = 11.84, df=1, $p < 0.01$.

7.2(b):

Nights (out of 12)	Probability		Number of larvae: Expected (Prob. x 30)		Observed (out of 30)
0	.0412		1.24		3
1	.1506		4.52		6
2	.2521		7.56		5
3	.2557		7.67		9
4	.1751		5.25		1
5	.0853		2.56		2
6	.0303		0.91		1
7	.0079		0.24		3
8	.0015		0.05		0
9	.0002	P=0.0399	0.01	EXP=1.21	0
10	≈ 0		≈ 0		0
11	≈ 0		≈ 0		0
12	≈ 0		≈ 0		0

G test for comparison of observed and expected numbers (bracketed) = 4.25, df=1, $p<0.05$.

A, and nights 6 – 12 inclusive for colony B, as shown by the brackets in the tables. A G test then gives a value of 11.84 (1 d.f., $p< 0.01$) for colony A, and 4.25 (1 d.f., $p<0.05$) for colony B. The null hypothesis H1 can therefore be rejected, i.e. some larvae occur in leading positions more frequently than would be expected by chance alone.

A further test with larvae <u>not</u> in leading positions is of interest because of the large number of observed larvae which did not occur in leading positions at all, or did so only once (Table 7.2a). A χ^2 test of the observed versus expected number of such larvae for 0 plus 1 nights gives $P = 6.64$ (1d.f., $p<0.01$) for colony A, and $P = 0.67$ (N.S.) for colony B. Clearly, colony A had many more larvae not occuring in leading positions than expected.

A 'leader' can then be defined as a larva occurring in a leading position more frequently than would be expected by chance alone; that is on more than 4 nights for colony A, and more than 5 nights for colony B (at $P<0.05$). Using this definition, there were 9 leaders in colony A, and 4 leaders in colony B, indicating that leaders made up 13-19% of the 2 colonies.

Similar analyses were made using (i) leading positions defined by the front 3 (or 4) positions only, and (ii) leading positions defined by the last 3 (or 4) positions only. The probabilities, and observed and expected numbers of occurrences of larvae in leading positions on 0,1,2,...n nights are given in Table 7.3a for criterion (i), and in Table 7.3b for criterion (ii). Expected numbers of larvae were again calculated with the binomial theorem, as 3.33 per night in colony A, and 3.50 in colony B.

Comparisons were made between observed and expected numbers of larvae for 0 and ≥ 1 nights (pooled). For colony A, G(i) = 7.22 (1 d.f., $p< 0.01$), and G(ii) = 12.72 (1 d.f., $p< 0.001$). For colony B, G(i) = 5.81 (1 d.f., $p< 0.05$), and G(ii) = 0.18 (1 d.f., N.S.). These results support the previous finding that 'leaders' occur inter-changeably at either end of the colony.

Table 7.3: The numbers of observed and expected occurrences of larvae in leading positions on 0,1,2...n nights for (a) leading positions defined by the front 3 (or 4) positions only, and (b) leading positions defined by the last 3 (or 4) positions only.

7.3(a):

Nights (out of n)	Observed (Col.A)	Expected (Col.A)	Observed (Col.B)	Expected (Col.B)
0	25	15.59	13	6.77
1	12	17.85	5	10.73
2	2	9.54	6	7.79
3	1	3.15	2	4.32
4	4	0.72	2	1.02
5	2	0.12	1	0.22
6	1	0.02	1	0.03
7	0	0	0	0
8	0	0	0	0

G(A) test for comparison of observed and expected numbers (bracketed) = 7.22, df=1, $p<0.01$.

G (B) test for comparison of observed and expected numbers (bracketed) = 5.81, df=1, $p<0.05$.

7.3(b):

0	28	15.59	8	6.77
1	7	17.85	11	10.73
2	5	9.54	6	7.79
3	3	3.15	4	4.32
4	1	0.72	0	1.02
5	1	0.12	0	0.22
6	0	0.02	0	0.03
7	2	0	1	0
8	0	0	0	0

G(A) test for comparison of observed and expected numbers (bracketed) = 12.72, df=1, $p<0.001$.

G(B) test for comparison of observed and expected numbers (bracketed) = 0.18, df=1, N.S.

4. **H2** : a larva recorded as leading on any night, say n(0), is no more likely to lead again on subsequent nights than would be expected by chance alone.

The probabilities, and the observed and expected numbers of larvae which occurred in leading positions on 0,1,2,...n nights are given for colonies A and B for n = 5 in Table 7.4a and n =10 in Table 7.4b.

Comparisons of the observed and expected numbers of larvae were made for the numbers of nights bracketed in the table. For Colony A, G (5 nights) = 24.54 (1d.f., $P < 0.001$) and G (10 nights) = 8.10 (1d.f., $P < 0.01$). For Colony B, G (5 nights) = 4.30 (1d.f., $P < 0.05$) and G (10 nights) = 2.98 (1d.f., N.S.). The more significant comparisons in colony A are probably due to it being a larger colony and being observed for a longer period of time. The results nevertheless show that some larvae ('leaders') occur in the leading positions more frequently than expected by chance alone, and that other larvae ('non-leaders') occur in leading positions less frequently than expected by chance alone, supporting the conclusions reached by testing H1.

The two subgroups of larvae can be clearly illustrated graphically, as in Figure 7.1, which is based on the observations made of larvae in colony A over 5 and 10 nights. The expected number of larvae is plotted against the number of reoccurrences of larvae in leading position on nights n(1) to n(5) and n(1) to n(10), and shows a marked drop as reoccurrences increase. On the other hand, the simultaneous plot of the observed numbers of larvae shows two distinct peaks; the first peak (few reoccurrences) represents 'non-leaders', and the second peak (many reoccurrences) represents 'leaders'.

Table 7.4: The probabilities, and the observed and expected numbers of larvae which occurred in the leading positions on 0,1,2,...n nights for colonies A and B for n=5 (a) and n=10 (b).

7.4(a):

Nights	Probability Col.A	Col.B	Exp.A (N=18)	Obs.A (N=18)	Exp.B (N=21)	Obs.B (N=21)
0	.4654	.2649	8.38	5	5.56	8
1	.3846	.4031	6.92	1	8.47	3
2		.2455		6	5.16	5
3	.1500		2.70	3		3
4		.0865		3	1.82	2
5				0		0

G(A) test for comparison of observed and expected numbers (bracketed) = 24.54, df=1, $p<0.001$.

G(B) test for comparison of observed and expected numbers (bracketed) = 4.30, df=1, $p<0.05$.

7.4(b):

Nights	Probability Col.A	Col.B	Exp.A (N=17)	Obs.A (N=17)	Exp.B (N=12)	Obs.B (N=12)
0	.2166	.0702	3.68	3	0.84	2
1	.3580	.2135	6.09	1	2.56	3
2		.2924		6	3.51	3
3		.2373		2	2.85	1
4	.4254	.1264	7.23	2	1.52	0
5				1		2
6		.0602		1	0.72	1
7				4		0
8				0		0

G(A) test for comparison of observed and expected numbers (bracketed) = 8.10, df=1, $p<0.01$.

G (B) test for comparison of observed and expected numbers (bracketed) = 2.98, df=1, N.S.

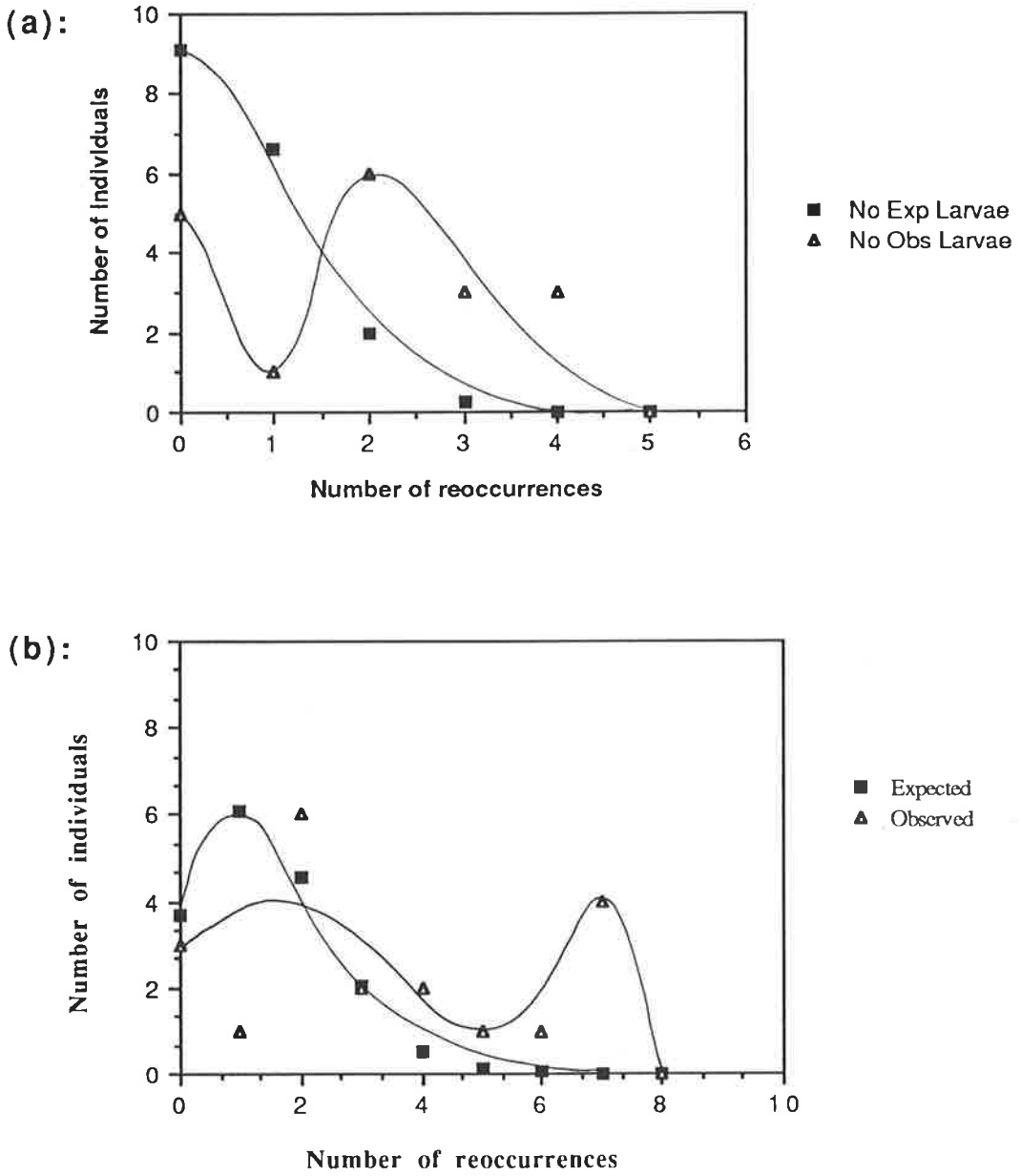


Figure 7.1: Observed and expected numbers of larvae plotted against the number of reoccurrences of larvae in leading positions over 5 nights (a) and 10 nights (b) of observation of colony A.

7.1.3.2 Experiment 2 : Manipulative relocation of larvae.

H3: Larvae remain in the positions into which they are placed following manipulation.

Table 7.5 gives the observed and expected numbers of occurences in lead positions of each subject larva over 5 nights following (i) placement into the centre of the colony and (ii) the nightly dispersal and reaggregation of the colony.

A comparison of the total observed and total expected occurences in leading and non-leading positions gives a G value of 68.05 (1 d.f., $p < 0.001$). H3 can therefore be rejected, and the occurrence of some larvae in 'leading' positions with greater frequency is therefore likely to be the result of genuine behavioural differences between larvae.

Table 7.5: The observed and expected number of occurences in lead positions of each subject larva over the next 5 nights following: (i) Placement into the centre of the colony, and (ii) The nightly dispersal and reaggregation of the colony.

	X		Y		Total
Day	(i)	(ii)	(i)	(ii)	
1	0	1	1	1	
2	1	1	1	1	
3	1	0	1	0	
4	1	1	0	1	
5	1	1	1	moulted	
Obs. leads	4	4	4	3	15
Exp. leads	0	0	0	0	1*
Obs. non-leads	1	1	1	1	4
Exp. non-leads	5	5	5	4	18*

G test for comparison of observed and expected numbers (bracketed) = 68.05, df=1, $p<0.001$.

*Calculated on the basis of 5% by chance alone (N=19).

Discussion

The results from this study clearly indicate that some larvae in a colony do most of the 'leading' when the larvae disperse to feed at night. Two subgroups of larvae are evident; 'leaders', which occupy leading positions frequently, and 'non-leaders', which occupy leading postions seldom or not at all.

Leaders may occur either at the front end or the tail end of a trail of larvae; the front end and tail end positions are interchangeable in the sense that leaders at the front end on one night may appear at the tail end on some subsquent nights and vice versa. This may indicate that leadership behaviour is not an accidental bi-product of the larvae's position within the colony, because if leaders are removed from the ends of the colony and placed in the middle, they are quick to regain a position at one end (Experiment 2).

This may indicate either that leaders are capable of leading both by 'pulling' or by 'pushing', or that a leader may lead the colony out from the front, and later lead it back, ending up at the opposite end of the colony.

The existence of a 'leader' subgroup within larval sawfly populations has significant ecological implications. Firstly, it may offer an explanation for the lack of viability of small colonies. In the closely related *Perga affinis* Kirby, colonies of less than about 20 individuals become weak and feed less efficiently, thus prolonging larval development into the unfavourably high temperatures of early summer (Carne, 1969). Similarly, larvae of *P. dorsalis* which occurred in small groups during this study were observed to weaken and wander away from the colony, or fall off and die on the ground. The poor performance of small colonies could be due to the lack of a critical number of 'leaders' required for a colony to remain cohesive and forage efficiently.

Secondly, the 'leader' subgroup may be differentially exposed to parasitoids and predators. In the Adelaide region, three major parasitoids attack *P. dorsalis*. They are: (1) The trigonalyid wasp, *Taeniogonalos venatoria* Riek, which oviposits along the leaf margins. Sawfly larvae become parasitised by consuming the eggs with foliage

(Chapter 3). (2) Tachinid flies, *Froggatimyia* spp., which 'stalk' the sawfly larvae on foot from around the colony, ovipositing on the peripheral individuals. (3) An ichneumonid wasp, *Westwoodia* sp., which walks over groups of young larvae, piercing them with the ovipositor, apparently indescriminately (see Section 7.2). Although 'leaders' arrive at the leaf margin and commence feeding before other larvae, all larvae feed initially upon fresh sections of leaf margin, rendering them equally susceptible to parasitisation by trigonalyids. The tachinids are therefore the only parasitoids which are obviously more likely to oviposit on 'leaders', as the latter occupy the fore and aft positions in the colony. However, higher 'attack' rates by tachinids on leaders do not necessarily equate to higher parasitisation rates, because leaders may be physiologically more capable of encapsulating tachinid larvae.

The relationship between leadership behaviour and predation is more difficult to consider, because no predators of pergid larvae have been observed in the Adelaide region. However, pergid larvae elsewhere have been recorded from the stomach contents of several birds, including the white-faced heron, bush thick-knee, gang-gang cockatoo, Pt. Lincoln ring-neck, several cuckoos, and the tawny frogmouth (Barker and Vestjens, c1989). A nocturnal predator, such as the tawny frogmouth, may well attack leading larvae more frequently as a result of leaders being the first to move. Other birds are less likely to prey differentially; cockatoos, for example, feed on whole colonies in true banquet fashion (Carne, pers. com.). Although there are no other significant predators of Australian pergid larvae, Tostowaryk (1971) describes predation upon similarly gregarious diprionid sawfly larvae in Canada. The larvae at the periphery of the colonies (possibly also leaders) are preferentially preyed upon by a pentatomid. These same larvae, however, are also those which have been preferentially parasitised by a peripherally attacking tachinid parasitoid. These peripheral (? leader) larvae thus absorb what would otherwise amount to twice the population mortality.

Finally, recall the 'active type 1' larvae of *Malacosoma californium pluviale* which give rise to more highly dispersing and more fecund adults (Wellington, 1957, 1977). With such individuals, the species is able to repopulate areas in which it has become temporarily extinct

after several years of particularly harsh spring weather. The insect is thereby able to exploit its immediate surroundings, at the same time as locating new climatic refuges. It would be interesting to determine whether a similar survival strategy exists in pergid sawflies. Such a survival strategy would complement the other major survival strategy of pergids in harsh, variable environments, namely the occurrence of diapause for up to 4 seasons in varying proportions of the population.

7.2. Cycloalexy in *Perga dorsalis* Leach

7.2.1 Introduction

The ring defence strategy adopted by larval chrysomelids (Chrysomelidae: Coleoptera) has recently been formally described as cycoalexy (Vasconcellos-Neto and Jolivet, 1988). The strategy is also adopted by some tenthredinid sawflies, and is described here for the larvae of *Perga dorsalis* Leach (Pergidae: Hymenoptera). The behaviour is discussed in relation to the parasitoids of *P. dorsalis* in the Adelaide region.

7.2.2 Materials and Methods

The behaviour of *P. dorsalis* larvae and their parasitoids was observed between 1986 and 1988, both on reared material in the laboratory (Waite Institute), and in the field (Adelaide region). Notes were made on the interaction of *P. dorsalis* larvae with their parasitoids, and all parasitoids were collected for identification. The effect of cycloalexy on predators could not be studied, as there are no known predators of *P. dorsalis* in the Adelaide region (see Section 7.1.4).

7.2.3 Results

(i) Circular colony formation

In the Adelaide region of South Australia, adult female *P. dorsalis* oviposit in autumn (February-March), and the gregarious larvae feed as colonies during the winter (see Section 3.4.3). Eggs are oviposited in groups of 40 to 50; they are inserted into the mesophyll of *Eucalyptus* leaves and form a pod adjacent to the midrib. After an incubation period of approximately 30 days, 2 to 4 first instar larvae (? leaders) chew their way out through the leaf cuticle. The remaining larvae follow them out through these emergence holes, and all larvae aggregate on the underside of the leaf. The first arriving larvae assume a ring formation within minutes, and are joined by the remaining larvae as they emerge, usually within one hour. The larvae move to the leaf margins to feed during the night, and reassemble in their circular formation before dawn (Fig.7.2). In the second or third instar the larvae can no longer fit under a leaf in this formation; they therefore interlock in a cylindrical mass around a twig or branch

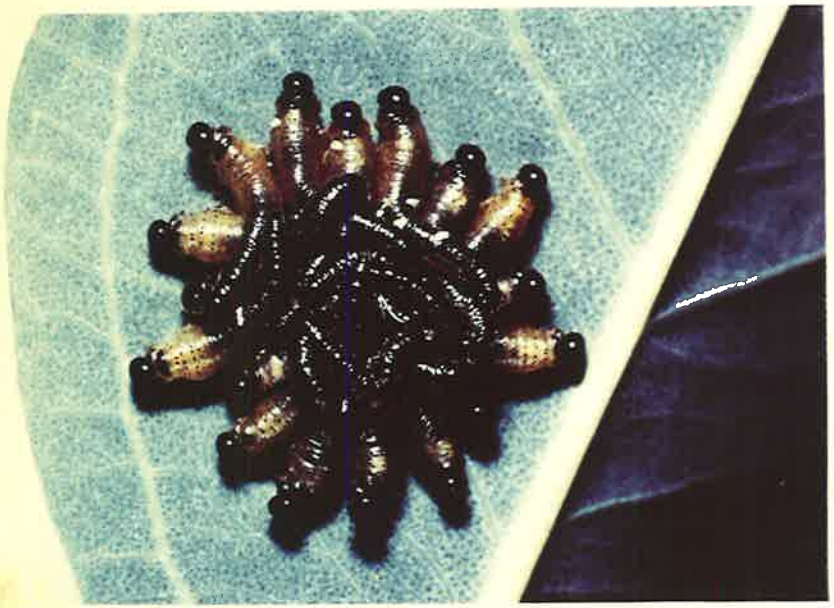


Figure 7.2: Second instar *P. dorsalis* larvae demonstrating cycloalexy. White tachinid eggs can be seen on the thoracic cuticles of some larvae.

Figure 7.3: Regurgitation of sequestered *Eucalyptus* oil by a manually disturbed *P. dorsalis* larva. The larave react in unison to natural disturbances.

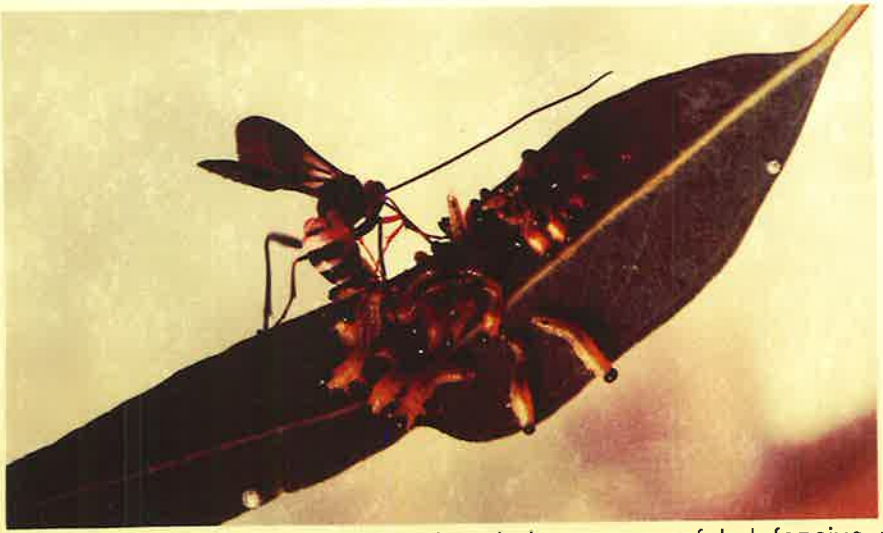


Figure 7.4: *P. dorsalis* larvae leaving their unsuccessful defensive ring in response to and ovipositing ichneumonid (*Westwoodia* sp.).

Figure 7.5: *P. dorsalis* larva gripping the ovipositor of an ichneumonid with its mandibles, rendering the parasitoid incapable of further attack.

instead. In the final instars this mass is often found on the main trunk, and may be made up of several coalesced colonies from the same tree. Some coalesced colonies were observed to contain more than one species of sawfly in the field (*P. dorsalis* and *Pergagrapta condei* Benson).

(ii) Cycloalexy

The circular formation adopted by the early instars is a ring of larvae with their heads facing outwards. If the group is large, the larvae form concentric circles, with the abdomens of the outer ring interdigitating with the heads and thoraces of the inner ring. If disturbed, the larvae rear up, raising their heads and abdomens off the substrate. With severe disturbance, they also release their foreleg grip on the substrate, and approximate their head and abdomen in a 'U' shape (Fig. 7.3). In this manner the oesophageal diverticulum is compressed within the thorax, and a drop of its contents is ejected from the oral cavity (Tait, 1962). This drop is the most prominent part of the 'U' shaped larva, and may in part be smeared onto the dorsal abdomen, which is flicked repeatedly. The drop is composed of oils which are chromatographically identical to those contained in the *Eucalyptus* leaves on which the larvae feed, but the means by which these oils are sequestered in the diverticulum in not known (Morrow *et al.*, 1976). At cold temperatures the oil is highly viscous, and remains adherent to the mouthparts. On hotter days the oil can be very liquid and may eject some 10-15cm if the larvae rear up violently (hence the common name 'spitfire grubs'). The oil is an effective deterrent to potential predators such as ants, birds and mice (Morrow *et al.*, 1976). The larvae therefore probably adopt this circular pattern, in which they regurgitate in unison, specifically for the purpose of defence. The behaviour therefore fulfills the critera for cycloalexy, as described by Vasconcellos-Neto and Jolivet (1988).

Once the disturbance has passed, the larvae actively re-swallow the regurigitated oil. Oil which may have been 'spilled' onto the substrate (leaf or branch) is also re-swallowed. Larvae were observed to engage in mutual grooming, and cleaned 'spilled' oil off the head capsules of fellow larvae with their mouth parts. In addition to saving the oil to repel further attacks and later to use it in cocoon

construction, mutual grooming may prevent 'spilled' oil from sealing over ecdysial lines.

(iii) Cycloalexy in relation to the behaviour of parasitoids

In the Adelaide region, *P. dorsalis* is associated with a complex of 3 parasitoids. They are all larval-pupal parasitoids, attacking early (mainly second) instar larvae, and emerging from the host cocoons. These parasitoids use the following oviposition behaviours, which overcome the circular defensive strategy of their hosts:

(a) *Froggatimyia* spp. (Tachinidae: Diptera). There are at least 3 species (B. Cantrell, pers. comm.) with superficially identical behaviours. A fly 'stalks' the host larvae, approaching very slowly on foot, and stopping for several minutes should the larvae show any sign of disturbance. The fly works its way close enough to oviposit on the larvae; the abdomen of the fly is then curled through its front legs and an egg is deposited on the cuticle of the *P. dorsalis* larva. *Froggattimyia* spp. often walks very close to or even on top of the larvae, without eliciting a defence reaction.

(b) *Westwoodia* sp. (Ichneumonidae: Hymenoptera) This large wasp (20-25mm) locates a colony, straddles the group of larvae, and starts to oviposit into them. At first, the larvae rear up and regurgitate *Eucalyptus* oil. *Westwoodia* sp. has a relatively long gaster, ovipositor and legs, and the oil does not reach the head or body of the parasitoid. *Westwoodia* is apparently unaffected by the defensive behaviour of *P. dorsalis* larvae, and continues to oviposit into the larvae. After oviposition has continued for a few seconds, the larvae break formation and disperse on the leaf (Fig. 7.4). The ichneumonid sometimes follows individual larvae, probing after them with the ovipositor. When so persued, the sawfly larvae may resort to biting. This can be a very effective, although probably suicidal, means of protecting the colony should a larva sieze and hold the ovipositor of the parasitoid (Fig. 7.5).

(c) *Taeniogonalos venatoria* (Trigonalyidae: Hymenoptera). As demonstrated in Chapter 3, *T. venatoria* oviposits on foliage, and depends on the young host larvae to consume the parasitoid eggs with the foliage on which they were laid. The defensive ring of

regurgitating *P. dorsalis* larvae is thus never encountered by the parasitoid.

7.2.4 Discussion

Cycloalexy has been described here in the early instars of *P. dorsalis*. Similar behaviour is recorded for the closely related *P. affinis* (Carne, 1962). In related genera, cycloalexy occurs in *Pergagrapta condei* Benson (personal observation) and in *Pseudoperga lewisii* Westwood (Lewis, 1837). This defensive strategy is also widespread among other insects species (Jolivet *et al.*, 1991). Although cycloalexy in pergid larvae is demonstrably effective against polyphagous predators (Morrow *et al.*, 1976; Sillén-Tullberg, 1990), it does not appear to protect *P. dorsalis* larvae from specialised parasitoids.

CHAPTER 8

LEAF PETIOLE CHEWING AND THE SABOTAGE OF INDUCED DEFENCES.

CHAPTER 8

This chapter has been published as "Leaf petiole chewing and the sabotage of induced defences" by P. Weinstein (1990), Oikos, 58; 231-233. A copy of this publication is attached herewith. The raw data are given in Appendix 6.

Weinstein, P. (1990). Leaf petiole chewing and the sabotage of induced defences. *Oikos, 58*(2), 231-233).

GENERAL DISCUSSION

General Discussion

In this study, the oviposition strategy of *T. venatoria* was examined in relation to possible defensive strategies in the larvae of its host *P. dorsalis*; these were diapause, colony behaviour and petiole chewing. The strategies did not appear to affect the rate of parasitism by *T. venatoria,* so either this trigonalyid has overcome the defensive behaviours of its host, or the defensive behaviours of *P. dorsalis* have evolved in response to the presence of other parasitoids and predators. The relationship between pergid defences and trigonalyid attack can therefore be considered in terms of an evolutionary 'arms race' (Dawkins and Krebs, 1979; Austin 1985), similar to the arms race proposed for the co-evolution of plants and insect herbivores (Ehrlich and Raven, 1967). This discussion examines the behaviour of *P. dorsalis* and its parasitoids within such a framework and comments on the implications of the behaviour of both host and parasitoids for the rational selection of potential biological control agents for *Perga* spp.

(i) Trigonalyid Oviposition strategy and host diapause. The eonymph of *P. dorsalis* is well protected from parasitoids during diapause, both by *Eucalyptus* oils with which it constructs its cocoon, and by the 40cm or so of soil under which it is burried. *T. venatoria* overcomes these barriers by accessing the hosts at the larval stage (larval-pupal parasitoid).

Prolonged diapause, lasting 2 or more years (seasons), as demonstrated in *P. dorsalis* (Chapter 6), can also be an effective means for a host to avoid parasitoids; when host and parasitoid life cycles are synchronised, prolonged host diapause can avert a potential buildup of parasitoids. In many host insects, prolonged diapause

ensures the survival of at least some part of the population when densities of both host and parasitoid are high (Danks, 1987; Hanski, 1988). There is a potential selective advantage to the host in being able to 'disperse in time' by undergoing prolonged diapause; this selective advantage can only be actualised, however, if the diapausing stage is not susceptible to partasitoids. The selective advantage is lost if, as with *T. venatoria*, the parasitoid is already within the diapausing host. Furthermore, *T. venatoria* does not 'lose' its host in time when the latter undergoes prolonged diapause. By virtue of the first instar larva of *T. venatoria*, which completes development only once the host has pupated, *T. venatoria* effectively 'disperses in time' together with the host, and can emerge from host cocoons over 3 years old (Section 3.4.3).

Although not studied in detail, ichneumonid (*Westwoodia* sp.) and tachnid (*Froggatimyia* spp.) parasitoids of *P. dorsalis* in the Adelaide region are also larval-pupal parasitoids, and also appear to only complete development in pupating hosts (Section 7.2.). A selection pressure for prolonged diapause in *P. dorsalis* is therefore unlikely to result from this guild of parasitoids; and perhaps such a selection pressure is more likely to be produced by the variable climate and unpredictable resources, as discussed in Chapter 6.

(ii) Trigonalyid Oviposition strategy and 'leadership' behaviour in host larvae. Leadership behaviour (Section 7.1) results in the increased exposure of 'leading' larvae to tachinids (because tachinids oviposit on individuals furthest from the centre of the colony), whereas exposure to ichneumonids and to *T. venatoria* is unaffected. The larvae of the western tent caterpillar, *Malacosoma californicum pluviale* (Dyar), also exhibit leadership behaviour, and leading larvae are known to be physiologically more robust (Wellington, 1977). If the leading larvae of *P. dorsalis* are similarly more robust, these larvae may be more capable of overcoming a parasitoid attack, for example by encapulating parasitoid eggs. On-the-other-hand, if leading larvae are physiologically as susceptible to parasitism as are non-leading larvae, then parasitoids would impose a significant selection pressure against the maintenance of a subgroup of leaders within the population.

The leading larvae of *M. californicum pluviale* also differ from other larvae within the colony by giving rise to highly fecund and widely dispersing adults. In the context of the variable environment in which this species is found, it is easy to see how the ecological advantages of maintaining a polymorphic population may outweigh (or balance) any negative selection pressure from parasitoids. In *P. dorsalis*, the attributes of adults which develop from leading larvae have not been studied. It is likely that these attributes are similar to those found in the *M. californicum pluviale*, because a selective advantage is necessary to maintain a subgroup of leaders in the population. Differential predation upon parasitised larvae, which in effect halves the total mortality in the population (Tostowaryk, 1971), is unlikely to account for such a selective advantage, because no predators of *P. dorsalis* were observed in South Australia. Further, predators are of only minor significance in New South Wales and Victoria, even when larvae are abundant (Carne, pers. comm.). It is not known if the larvae that demonstrate leadership behaviour are also those that are more likely to undergo prolonged diapause, but the search for such a correlation could provide an interesting avenue of investigation.

Finally, it cannot go without mention that diprionid sawflies show amazing convergence with *Perga* in that their larvae are gregarious, undergo prolonged diapause, and demonstrate defensive behaviours which involve sequestered host plant oils (Prop, 1960; Coppel and Benjamin, 1965). The larvae of diprionid sawflies may therefore also demonstrate leadership behaviour, and this possibility is worthy of investigation.

(iii) Trigonalyid oviposition strategy and cycloalexy in host larvae. The circular defense strategy (cycloalexy) exhibited by the larvae of *P. dorsalis* (Chapter 7.2) is widespread among insect species (Jolivet *et al.* 1991). Although cycloalexy is effective against predators (Morrow *et al.*, 1976; Sillén-Tullberg, 1990), its effect on parasitoids is less clearcut. Diprionid sawflies, as mentioned above, have many features in common with *Perga* spp., and colony members in gregarious diprionids also rear up and regurgitate sequestered host plant (Pinaceae) oils when threatened by predators (Prop, 1960). Tripp (1960, 1962) describes the behavioural mechanisms whereby parasitoids overcome these defenses in *Neodiprion swainei* Middleton

(Diprionidae). Two tachinids (*Exenterus diprionis* Rohwer and *Spathimeigenia spinigera* Townsend) employed a combination of stealth and speed to avoid eliciting a defensive reaction, and two ichneumonids (*Olesicampe lophyri* Riley and *Lamachus* sp.), although eliciting a defensive reaction, remain at a 'safe' distance from the colony while ovipositing by virtue of their long abdomens. These behaviours are remarkably similar to those employed by *Froggatimyia* spp. and *Westwoodia* sp. to overcome cycloalexy in *P. dorsalis*. Certainly, both of those parasitoids succeed in parasitizing some larvae, but the influence of cycloalexy or the rate of parasitism is unknown. *T. venatoria*, however, is not directly affected by cycloalexy, because host contact is not required for oviposition. Furthermore, experiments on host selection behaviour by the adults (Chapter 5) demonstrated that the presence of *P. dorsalis* larvae neither deterred nor enhanced the selection of oviposition substrata by *T. venatoria*. Therefore it is unlikely that *T. venatoria* detects even the volatile components of the oils regurgitated by *P. dorsalis* larvae.

Although there is at present no interaction between *T. venatoria* and its host larvae in cycloalexy, trigonalyids may have been affected by cycloalexy in a host-parasitoid arms race during their evolutionary past. Several authors have suggested that the ancestral hosts of trigonalyids were sawflies (for example Cooper, 1954; Malyshev, 1968) and the presence of trigonalyids in predominantly woodland habitats can be regarded as circumstantial evidence supporting such an association (Popov, 1945). If in evolutionary history sawflies developed gregariousness and cycloalexy before ancestral trigonalyids had abandoned host selection (See Introduction, p.2), cycloalexy might have been a significant factor in driving trigonalyids to oviposit on foliage.

(iv) Trigonalyid oviposition strategy and petiole chewing in host larvae. In an experiment carried out in the absence of parasitoids, *P. dorsalis* larvae which were prevented from petiole chewing weighed more than did controls (Chapter 8). This result was unexpected because heavier insect larvae are usually fitter, in terms either of a reduced rate of mortality or of increased fecundity of the resultant adult, as is true for many caterpillars (Morris, 1965). So if larvae which chew petioles (in the usual way) are less fit than they would be

if they did not chew petioles, what then provides a countering positive selective pressure to maintain the habit of petiole chewing in the population? The following hypothesis is suggested: In colonies in which *P. dorsalis* larvae remove the physicochemical cues used by parasitoids in host selection, mortality due to parasitism is decreased. In parasitoid species which conform to Vinson's (1976) model of host selection, the task of locating host larvae would be made much more difficult by the removal of such cues. It might therefore be expected that the mortality due to tachinid and ichneumonid parasitoids of *P. dorsalis* would be decreased by petiole chewing, and that overall, a decrease in larval mortality in *P. dorsalis* would result.

T. venatoria, which did not respond to leaves that had been chewed by host larvae (Chapter 5), is unlikely to be affected by petiole chewing. Nevertheless, as discussed for cycloalexy, it is possible that the adoption of petiole chewing by the ancestral hosts of trigonalyids drove the parasitoid to oviposit on leaves when host larvae became increasingly difficult to locate.

(v) The management of sawfly populations. Compared to other parasitoids, the biology and oviposition strategy of trigonalyids at first appears to be very risky and inefficient; good biological control agents are usually highly species specific, demonstrate high searching capacity, and are multivoltine with long-lived adults (De Bach, 1964). However, as discussed in Chapters 6, 7 and 8, trigonalyid oviposition strategy overcomes many of the problems which would confront other potential parasitoids of *Perga* spp., particularly host defensive behaviours. When attempting to control a pest species with such highly developed defensive behaviours, a trigonalyid may therefore be a reasonable choice as a biological control agent. Any such trigonalyid would, however, need to be capable of development as a primary parasitoid; hyperparasitoids are usually detrimental to biological control programmes because they kill primary parasitoids which help to control the pest population.

As one trigonalyid which fulfills these criteria, *T. venatoria* may be worth considering for the control of *P. dorsalis* and other *Eucalyptus* defoliating *Perga* spp. (s.l.). In some areas of southeastern Australia, *T. venatoria* is the most significant mortality factor in populations of

Perga affinis Kirby, and over 50% of host larvae are parasitized (Carne, 1969). As the importance of hardwood plantations increases in Australia, and the acceptability of insecticide use decreases, it may become necessary to rely increasingly on trigonalyids to control the buildup of sawfly populations. As young trees in newly established woodlots are particularly susceptible to sawfly outbreaks, it may be advisable to make innoculative releases (Metcalf and Luckmann, 1985) of trigonalyids before sawflies become abundant in such areas.

APPENDIX 1

WEINSTEIN AND AUSTIN (1991).

The host relationships of trigonalyid wasps (Hymenoptera: Trigonalyidae), with a review of their biology and catalogue to world species, by P. Weinstein and A. D. Austin (1991), Journal of Natural History, 25, 399-433.

A reprint of the publication is attached inside the back cover of the thesis.

APPENDIX 2

RAW DATA AND ANALYSIS, CHAPTER 2.

Appendix 2.1

The occurrence (1) and non-occurrence (0) of P. dorsalis in 3 subsamples at each of 77 field sites. The presence of T. venatoria is denoted by (*). The field sites are listed (i) by average annual rainfall (mm) in decresing order of magnitude and (ii) alphabetically within each rainfall category.

Rainfall	Location	P. dorsalis	T. venatoria
>1200	Stirling	0	
		0	
		0	
1000-1200	Bridgewater	1	
		0	
		0	
	Uraidla	0	
		0	
		0	
800-1000	Clarendon	1	
		0	
		0	
	Echunga	1	
		1	
		0	
	Gumeracha	0	
		0	
		0	
	Hahndorf	1	*
		1	
		1	
	Lobethal	1	
		1	
		0	
	Meadows	1	
		1	
		0	
	Mt.Compass	1	*
		1	
		1	
700-800	Birdwood	1	
		0	
		0	
	Inman Valley	1	
		1	
		1	
	Macclesfield	0	
		0	
		0	
	Mt.Barker	1	
		1	
		0	
	Mt.Gambier	1	*
		1	
		1	
	Mt.Shank	1	*
		1	
		1	
	Myponga	1	
		1	
		1	
600-700	Baker Range	1	
		1	
		1	
	Clare	0	
		0	
		0	
	Pers Chase K.	1	
		0	
		0	
	Glen Osmond	1	*
		1	
		1	
	Happy Valley	1	*
		1	
		0	
	Magill	1	
		1	
		0	
	McLaren Vale	1	*
		1	
		1	
	Mitcham	1	*
		1	
		1	
	Mt.Crawford	1	
		1	
		0	

Rainfall	Location	P. dorsalis	T. venatoria
600-700	Parkside	1	*
		1	
		1	
	Penola	1	*
		1	
		1	
	Teatree Gully	1	*
		1	
		1	
	Watervale	1	
		0	
		0	
	Williamstown	1	
		0	
		0	
500-600	Adelaide	1	
		1	
		1	
	Angaston	0	
		0	
		0	
	Auburn	0	
		0	
		0	
	Brighton	1	*
		1	
		1	
	Lyndoch	1	
		1	
		0	
	Mintaro	0	
		0	
		0	
	Naracoorte	1	*
		1	
		1	
	Nara'caves	1	
		0	
		0	
	Noralunga	1	
		1	
		1	
	North Adelaid	1	*
		1	
		1	
	Riverton	0	
		0	
		0	
	St. Peters	1	
		1	
		1	
	Tanunda	1	
		1	
		0	
	Unley	1	
		1	
		1	
	Yankalilla	0	
		0	
		0	
400-500	Airport	1	
		1	
		1	
	Burra	0	
		0	
		0	
	Coonalpyn	1	
		0	
		0	
	Edithburg	0	
		0	
		0	
	Gawler	1	
		1	
		1	
	Glenelg	1	*
		1	
		1	

Rainfall	Location	P. dorsalis	T. venatoria
400-500	Hamley Bridge	0	
		0	
		0	
	Kapunda	0	
		0	
		0	
	Keith	0	
		0	
		0	
	Kingscote	0	
		0	
		0	
	Meningie	0	
		0	
		0	
	Minlaton	0	
		0	
		0	
	Pt. Adelaide	1	
		1	
		1	
	Roseworthy	0	
		0	
		0	
	Salisbury	1	
		1	
		0	
	Truro	0	
		0	
		0	
	Virginia	0	
		0	
		0	
	Warooka	0	
		0	
		0	
	Yorketown	1	
		0	
		0	
350-400	Kadina	0	
		0	
		0	
	Langhorne Cr.	1	
		0	
		0	
	Mallala	0	
		0	
		0	
	Moonta	0	
		0	
		0	
	Tailem Bend	0	
		0	
		0	
	Two Wells	0	
		0	
		0	
	Wellington	1	
		0	
		0	
<350	Murray Bdge.	1	
		0	
		0	
	Pt. Augusta	0	
		0	
		0	
	Pt. Pirie	0	
		0	
		0	
	Ouorn	0	
		0	
		0	
	Woomera	0	
		0	
		0	

Appendix 2.2

ANOVA table for 1-factor analysis of variance of the mean fraction of samples with P. dorsalis larvae in relation to average annual rainfall. The categories of rainfall are those given in Table 2.1 and Appendix 2.1.

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Between gps	8	13.4	1.7	8.48	<0.001
Within gps	222	43.8	0.2		
Total	230	57.2			

Appendix 2.3

The observed and expected number of trees with P. dorsalis present or absent in each of 12 categories of 4 species of tree x 3 heights, to test the hypothesis that the presence of P. dorsalis, the species of tree, and the height of tree are mutually independent on manipulated trees.

OBSERVED

	P. dorsalis present			P. dorsalis absent			
Height of tree	<2m	2-6m	>6m	<2m	2-6m	>6m	Row totals
E. camaldulensis	20	37	6	21	98	124	306
E. leucoxylon	6	8	0	33	29	12	88
E. other	8	4	0	1	8	8	29
E. torquata	5	9	0	16	29	14	73
Table totals		103			393		
Column totals		110	222	164			n = 496

EXPECTED

	P. dorsalis present			P. dorsalis absent		
Height of tree	<2m	2-6m	>6m	<2m	2-6m	>6m
E. camaldulensis	14.09	28.45	21.01	53.77	108.52	80.15
E. leucoxylon	4.05	8.18	6.04	15.46	31.21	23.05
E. other	1.34	2.7	1.99	5.1	10.28	7.6
E. torquata	3.36	6.79	5.01	12.83	25.89	19.12
Table totals		103			393	
Column totals		110	222	164		

Chi-Square for 3-dimensional contingency table analysis = 119.48; d.f. = 17; P < 0.001

Appendix 2.4

The observed and expected numbers of trees with P. dorsalis present or absent in each of 12 categories of 4 species of tree x 3 heights, to test the hypothesis that the presence of P. dorsalis is independent of the species of tree and the height of tree on manipulated trees.

OBSERVED

	E. camaldulensis			*E. leucoxylon*			*E. other*			*E. torquata*			*total*
Height of tree	<2m	2-6m	>6m	<2m	2-6m	>6m	<2m	2-6m	>6m	<2m	2-6m	>6m	
P. dorsalis present	20	37	6	6	8	0	8	4	0	5	9	0	103
P. dorsalis absent	21	89	124	33	29	12	1	8	8	16	29	14	393
													496

EXPECTED

	E. camaldulensis			*E. leucoxylon*			*E. other*			*E. torquata*		
Height of tree	<2m	2-6m	>6m	<2m	2-6m	>6m	<2m	2-6m	>6m	<2m	2-6m	>6m
P. dorsalis present	14.1	28.5	21	4.05	8.18	6.04	1.34	2.7	1.99	3.36	6.79	5.01
P. dorsalis absent	53.8	109	80.2	15.5	31.2	23.1	5.1	10.3	7.6	12.8	25.9	19.1

Chi-square for 3-dimensional contingency table analysis = 145.16; d.f. = 11; $P < 0.001$

Appendix 2.5

The observed and expected numbers of trees with P. dorsalis larvae present or absent in each of 3 height categories of E. camaldulensis, to test the hypothesis that the presence of P. dorsalis is independent of tree height in this species. G-values (log-likelyhood ratio) are also given for the pairwise comparison of height categories.

OBSERVED - E. camaldulensis only

Height of tree	Present	Absent
< 2m	20	21
2-6m	37	98
> 6m	6	124

EXPECTED - E. camaldulensis only

Height of tree	Present	Absent
< 2m	8.44	32.56
2-6m	27.79	107.21
> 6m	26.76	103.24

Total Chi-square = 44.058, df = 2, p <0.0001

Height cat'ies	G-value	Probability
<2m vs 2-6m	6.30	<0.01
<2m vs >6m	40.33	<0.001
2-6m vs >6m	27.81	<0.001

Appendix 2.6 (a)

The observed and expected numbers of trees with P. dorsalis larvae present or absent for each of 4 species of Eucalyptus <2m tall, to test the hypothesis that the presence of P. dorsalis is independent of tree species in this height category. G-values (log-likelyhood ratio) are also given for the pairwise comparison of species.

OBSERVED < 2m trees only

Species of tree	Present	Absent
E. camaldulensis	20	21
E. leucoxylon	6	33
E. other	8	1
E. torquata	5	16

EXPECTED < 2m trees only

Species of tree	Present	Absent
E. camaldulensis	14.54	26.46
E. leucoxylon	13.83	25.17
E. other	3.19	5.81
E. torquata	7.45	13.55

Total Chi-square = 22.52, df = 3, p <0.0001

Species	G-value	Probability
E.c. vs E.l.	10.59	<0.01
E.c. vs E.o.	5.50	<0.05
E.c. vs E.t.	3.75	0.06
E.l. vs E.o.	18.18	<0.001
E.l. vs E.t.	0.63	0.42
E.o. vs E.t.	11.72	<0.01

Appendix 2.6 (b)

The observed and expected numbers of trees with P. dorsalis larvae present or absent for each of 4 species of Eucalyptus 2-6m tall, to test the hypothesis that the presence of P. dorsalis is independent of tree species in this height category.

OBSERVED - 2 to 6m trees only

Species of tree	Present	Absent
E. camaldulensis	37	98
E. leucoxylon	8	29
E. other	4	8
E. torquata	9	29

EXPECTED - 2 to 6m trees only

Species of tree	Present	Absent
E. camaldulensis	35.27	99.73
E. leucoxylon	9.67	27.33
E. other	3.14	8.86
E. torquata	9.93	28.07

Total Chi-square = 0.944, df = 3, p = 0.81; Not significant.

Appendix 2.6 (c)

The observed and expected numbers of trees with P. dorsalis larvae present or absent for each of 4 species of Eucalyptus >6m tall, to test the hypothesis that the presence of P. dorsalis is independent of tree species in this height category.

OBSERVED > 6m trees only

Species of tree	Present	Absent
E. camaldulensis	6	124
E. leucoxylon	0	12
E. other	0	8
E. torquata	0	14

EXPECTED > 6m trees only

Species of tree	Present	Absent
E. camaldulensis	4.76	125.24
E. leucoxylon	0.44	11.56
E. other	0.29	7.71
E. torquata	0.51	13.49

Total Chi-square = 1.629, df = 3, p = 0.65; Not significant.

APPENDIX 3

RAW DATA, CHAPTER 3.

A 3.1

Percentage eclosion in response to irrigation with various fluids : 10 different combinations of fluids (constituting the 10 treatments) are given, in the chronological order in which they were applied to n eggs in each replicate. The number of eclosions (N) were counted 7 days after the start of treatment. The controls A and B were irrigated with the same solutions used in treatments 9 and 4 respectively, but the eggs had not been manipulated with a needle. Eggs in treatment 1 were not irrigated with any fluids other than the saline into which all eggs were originally placed.

Date start	Treat-ments	Repli-cate	Homo-genate	pH 7.5 saline	Trypsin 2%	pH 6.0 saline	Chymo-tryp 2%	CO_2 30 min	Date count	n eggs	N eclosed
20.6	1	1							27.6	30	3
& 21.6	1	2								30	2
	2	1	+							30	1
	2	2	+							30	0
	3	1		+	+					30	0
	3	2		+	+					30	0
	4	1				+	+			30	4
	4	2				+	+			30	4
	5	1						+		30	0
	5	2						+		30	0
	6	1	+					+		30	0
	6	2	+					+		30	0
	7	1		+	+			+		30	0
	7	2		+	+			+		30	0
	8	1				+	+	+		30	2
	8	2				+	+	+		30	0
15.7	1	3							24.7	30	4
	1	4								30	3
	9	1				+				30	2
	9	2				+				30	4
	4	3				+	+			30	5
	4	4				+	+			30	3
	10	1					+			30	1
	10	2					+			30	0
4.8	9	3				+			9.8	30	3
	9	4				+				30	1
1.9	9	5				+			6.9	30	0
	9	6				+				30	1
30.6	A	1				+			7.7	30	0
	A	2				+				30	1
	B	1				+	+			30	0
	B	2				+	+			30	0

APPENDIX 4

RAW DATA AND ANALYSIS, CHAPTER 5.

A 4.1.1

ANOVA table for 1-factor analysis of variance of the influence of 3 treatments on the percentage of 3 kinds of adults of T. venatoria selecting the arm with the odour source in the Y-tube olfactometer. The treatments and kinds of adults are given in Table 5.1.

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Between gps	7	771.5	110.2	1.49	0.29
Within gps	8	590.6	73.8		
Total	15	1362.1			

A 4.1.2

ANOVA table for 2-factor analysis of variance of trigonalyid type and odour source on the percentage of T. venatoria selecting the arm with the odour source in the Y-tube olfactometer. The treatments with naïve unfed T. venatoria have been omitted.

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Odour source*	2	87.5	43.8	0.57	0.59
Trig type; NF/E	1	252.1	252.1	3.27	0.12
Intereaction	2	304.2	152.1	1.97	0.22
Error	6	462.5	77.1		

* See section 5.3.1.1.

A 4.1.3

ANOVA table for 2-factor analysis of variance of trigonalyid type and odour source on the percentage of T. venatoria selecting the arm with the odour source in the Y-tube olfactometer. The treatments with odour source 3 (larvae on leaves) have been omitted.

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Odours 1 & 2*	1	0.5	0.5	0.01	0.94
Trig type 1-3*	2	50.0	25.0	0.29	0.76
Intereaction	2	404.2	202.1	2.38	0.17
Error	6	509.4	84.9		

* See section 5.3.1.1.

A 4.2.1

The trigonalyid type, replicate number, and number of trigonalyids entering each of 4 arms in the odour field olfactometer. The average number entering each arm is also given for both types of trigonalyids. The arms are coded as 1=odour source, 2=90° clockwise from odour source, 3=opposite odour source, 4= 90° anticlockwise from odour source.

Trigonalyid type	Replicate number	Number in replicate	No. of trigs (out of 12) choosing arm no.				Average no. per arm
			1	2	3	4	
Naïve fed	1	(12)*	2.25	3.75	3	3	
	2	(12)*	3	1.5	3.75	3.75	
	3	12	6	1	2	3	
	4	12	4	2	3	3	
	5	12	2	4	4	2	
	6	12	4	1	0	7	
Average no. (/12) in each arm:			3.54	2.21	2.62	3.62	3.00
Experienced	1	(12)*	3	3.75	2.25	3	
	2	(12)*	1.5	2.25	3	5.25	
	3	12	3	4	4	1	
	4	12	5	3	2	2	
	5	12	3	3	3	3	
	6	12	4	4	2	2	
Average no. (/12) in each arm:			3.25	3.33	2.71	2.71	3.00

(12)* denotes replicate in which 16 trigonalyids were used. The number entering each arm has been converted to a figure out of 12 to facilitate comparison between replicates.

A 4.2.2

ANOVA table for 2-factor analysis of variance of trigonalyid type and arm number (odour source) on the number (out of 12) of trigonalyids exiting through each corner of the 4 arm odourfield olfactometer.

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Arm no. 1-4	3	3.7	1.2	0.73	0.54
Trig type; NF/E	1	0.0	0.0	0.02	0.90
Intereaction	3	5.4	1.8	1.05	0.38
Error	40	68.2	1.7		

A 4.3.1

The wind speed, odour source and type of trigonalyid used in the wind tunnel (Section 5.3.1.3), together with the number (N) and percentage of T. venatoria alighting on the odour source and the number of T. venatoria used in each trial (n). The last row gives the figures used to calculate the incidental 'background' oviposition rate.

Wind	Odour	Odour	Inexperienced unfed trig.			Inexperienced fed trig.			Experienced fed trig.		
cm/s	source*	group*	n	N	%	n	N	%	n	N	%
18.0	A	1	10	1	10.0	10	0	0.0	10	0	0.0
	C	2	6	0	0.0	12	0	0.0	6	0	0.0
41.3	A	1	10	1	10.0	10	0	0.0	12	1	8.3
	B	3	10	0	0.0	12	0	0.0	10	0	0.0
	C	2	6	0	0.0	10	1	10.0	10	1	10.0
	D	3	12	2	16.7	10	0	0.0	12	0	0.0
	E	3	6	0	0.0	6	0	0.0	10	0	0.0
	F	3	6	0	0.0	6	0	0.0	10	0	0.0
	G	3	6	0	0.0	6	0	0.0	10	0	0.0
	H	3	6	0	0.0	6	1	16.7	10	0	0.0
	I	3	4	0	0.0	6	0	0.0	10	0	0.0
66.3	A	1	10	0	0.0	10	0	0.0	12	0	0.0
	C	2	6	1	16.7	6	0	0.0	10	1	10.0
109.0	A	-	6	0	0.0	6	0	0.0	6	0	0.0
	C	-	6	0	0.0	6	0	0.0	12	0	0.0
41.3	Paper	B'ground	-	-	-	-	-	-	135	5	3.7

* See section 5.3.1.3

A 4.3.2

ANOVA table for 2-factor analysis of variance of trigonalyid type and odour group on the orientation rate of T. venatoria in the windtunnel. The treatments at windspeed 109.0cm/s have been omitted.

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Odour gp 1-3*	2	83.1	41.6	1.37	0.27
Trig type 1-3*	2	49.4	24.7	0.81	0.45
Interaction	4	76.7	19.2	0.63	0.64
Error	30	910.0	30.3		

* See section 5.3.1.3.

A 4.4

The height of tree, distance from tree at which trigonalyids were released (m), and the number and percentage of trigonalyids which flew to the trees. The probit of this percentage and the angle subtended from the point of release to the top of the trees are also given.

• Indicates no insects released at this distance.

Height of tree	m from tree	Number of trigonalyids released	Number of trigonalyids flew to tree	% to tree	probit of %	canopy angle
2m	10	10	9	90.0	6.28	11.3
	20	10	9	90.0	6.28	5.1
	25	14	6	42.9	4.82	4.1
	30	10	3	30.0	4.48	3.8
	35	•	•	•	•	•
	40	9	2	22.2	4.23	2.9
	50	•	•	•	•	•
	60	•	•	•	•	•
	70	•	•	•	•	•
	90	•	•	•	•	•
10m	10	6	6	100.0	8.72	45.0
	20	10	10	100.0	8.72	26.6
	25	15	8	53.3	5.08	21.8
	30	9	6	71.4	5.55	18.4
	35	10	5	50.0	5.00	16.0
	40	7	3	42.9	4.82	14.4
	50	•	•	•	•	•
	60	5	1	20.0	4.16	9.5
	70	•	•	•	•	•
	90	4	0	0.0	1.91	6.3
12m	10	8	6	75.0	5.67	50.2
	20	8	6	75.0	5.67	31.0
	25	13	8	61.5	5.31	25.6
	30	17	9	52.9	5.08	21.8
	35	12	6	50.0	5.00	18.9
	40	10	4	40.0	4.75	16.7
	50	7	3	42.9	•	3.0
	60	•	•	•	4.82	11.3
	70	•	•	•	•	•
	90	•	•	•	•	•
20m	10	9	8	88.9	6.23	63.4
	20	10	9	90.0	6.28	45.0
	25	10	8	80.0	5.84	38.7
	30	8	7	87.5	6.18	33.7
	35	10	10	100.0	8.72	29.7
	40	10	4	40.0	4.75	26.6
	50	8	4	50.0	5.00	21.8
	60	11	5	45.5	4.87	18.4
	70	12	5	41.7	4.80	16.0
	90	•	•	•	•	•

A 4.5.1

ANOVA table for 2-factor analysis of variance of trigonalyid type and leaf type on ln(residency time) of 2 types of T. venatoria on the leaves of E. camaldulensis and V. vinifera.

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Trig type; NF/E	1	0.4	0.4	0.49	0.50
Leaf E.c./V.v.	1	0.7	0.7	0.79	0.39
Intereaction	1	4.8	4.8	5.28	0.04
Error	12	10.9	0.9		

A 4.5.2(a)

A 5.5.2(b)

The trigonalyid type, leaf type, oviposition rate (eggs per metre per hour) and ln(oviposition rate) for (a) 6 different plant species and (b) 3 types of E. camaldulensis leaves on which the oviposition rate of T. venatoria was recorded.

Trigonalyid type	Leaf type	Replicate	Oviposition rate	ln(ovip.rate)
Naive fed	V. vinifera	1	10.49	2.3504
		2	6.67	1.8976
		3	8.58	2.1494
		4	2.43	0.8879
		5	1.57	0.4511
		6	4.34	1.4679
	E. camal-dulensis	1	0.56	-0.5798
		2	1.61	0.4762
		3	0.58	-0.5447
		4	7.27	1.9838
		5	9.32	2.2322
		6	1.88	0.6206
		7	1.05	0.0488
		8	0.83	-0.1863
		9	0.37	-0.9943
		10	0.02	-3.9120
		11	0.04	-3.2189
		12	0.46	-0.7765
		13	1.08	0.0770
		14	0.14	-1.9861
		15	0.02	-3.9120
	H. flavum	1	23.19	3.1437
		2	25.28	3.2300
		3	17.03	2.8350
	Cotoneaster	1	4.53	1.5107
		2	11.87	2.4740
		3	4.88	1.5851
	A. iteaphylla	1	2.21	0.7930
		2	1.82	0.5988
		3	2.66	0.9783
	A. piquata	1	0.00	*
		2	0.03	-3.5066
		3	0.00	*
		4	5.18	1.6448
		5	8.09	2.0906
		6	7.74	2.0464
Experienced	V. vinifera	1	1.94	0.6627
		2	1.19	0.1740
		3	1.10	0.0953
		4	3.00	1.0986
		5	3.00	1.0986
		6	2.10	0.7419
	E. camal-dulensis	1	1.22	0.1989
		2	0.77	-0.2614
		3	0.25	-1.3863
		4	2.02	0.7031
		5	2.06	0.7227
		6	0.46	-0.7765
		7	1.29	0.2546
		8	0.82	-0.1985
		9	0.88	-0.1278
	H. flavum	1	0.52	-0.6539
		2	0.47	-0.7550
		3	1.31	0.2700
		4	0.29	-1.2379
		5	2.50	0.9163
		6	0.60	-0.5108
	Cotoneaster	1	5.87	1.7699
		2	3.37	1.2149
		3	5.32	1.6715
	A. iteaphylla	1	0.42	-0.8675
		2	1.14	0.1310
		3	0.34	-1.0788
	A. piquata	1	0.03	-3.5066
		2	0.55	-0.5978
		3	0.01	-4.6052

Trigonalyid type	Leaf type	Replicate	Oviposition rate	ln(ovip.rate)
Naive fed	Young clean	1	1.64	0.4947
		2	0.80	-0.2231
	Old clean	1	0.59	-0.5276
		2	0.39	-0.9416
		3	7.85	2.0605
		4	10.29	2.3312
		5	2.21	0.7930
		6	1.27	0.2390
		7	0.83	-0.1863
		8	0.48	-0.7340
	Damaged	1	0.00	*
		2	1.31	0.2700
		3	0.14	-1.9861
		4	2.27	0.8198
		5	3.91	1.3635
		6	0.86	-0.1508
		7	0.00	*
		8	0.00	*
Experienced	Young clean	1	2.40	0.8755
		2	0.50	-0.6931
	Old clean	1	1.57	0.4511
		2	0.89	-0.1165
		3	2.40	0.8755
		4	0.22	-1.5141
		5	0.20	-1.6094
		6	1.42	0.3507
		7	0.89	-0.1165
		8	0.96	-0.0408
	Damaged	1	0.32	-1.1394
		2	0.37	-0.9943
		3	1.01	0.0100
		4	0.63	-0.4620
		5	0.18	-1.7148
		6	0.00	*
		7	0.41	-0.8916
		8	0.51	-0.6733
		9	0.18	-1.7148

A 4.5.3

ANOVA table for 2-factor analysis of variance of trigonalyid type and leaf type on ln(oviposition rate) of 2 types of T. venatoria on the leaves of 6 different species of plants.

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Trig type; NF/E	1	27.8	27.8	15.11	< 0.001
Leaf species*	5	56.8	11.4	6.17	< 0.001
Intereaction	5	39.5	7.9	4.29	< 0.005
Error	52	95.7	1.8		

* See section 5.5.1.2.

A 4.5.4

ANOVA table for 2-factor analysis of variance of trigonalyid type and leaf type on ln(oviposition rate) of 2 types of T. venatoria on 3 types of E. camaldulensis leaves.

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Trig type; NF/E	1	1.7	1.7	1.74	0.20
Leaf type*	2	2.1	1.1	1.07	0.36
Intereaction	2	0.8	0.4	0.39	0.68
Error	27	27.0	1.0		

* See section 5.5.1.2.

A 4.6.1

The residency time and oviposition behaviour of 3 types of T. venatoria on the leaves of 3 different species of plants. The presence of oviposition is denoted by "1", its absence by "0". Two treatments were omitted: T. venatoria experienced on Acacia were not observed on either A. piquata or V. vinifera.

Trigonalyid type	Leaf species	Residency time	Oviposition Pres/Abs
Exper'ed on Acacia	E.camal'sis	0.40	0
		0.03	0
		0.78	1
		0.25	0
		0.07	0
		0.03	0
		0.07	0
		1.98	0
		2.25	0
		6.25	1
		3.70	1
		6.52	1
		9.27	1
		8.72	1
		2.33	1
		5.28	1
		10.00	0
Exper'ed on E.camal'sis		8.00	0
		7.00	1
		10.00	0
		4.00	0
		2.00	0
		8.00	0
		4.00	1
		0.33	0
		10.00	1
		10.00	1
		7.00	1
		4.00	1
		5.00	1
		6.50	1
		1.50	0
	A.piquata	0.50	0
		2.00	0
		0.02	0
		2.63	0
		2.58	0
		1.03	0
		1.42	0
		3.00	1
		1.20	1
		5.50	1
		4.75	0
		5.30	1
		5.00	1
		4.00	0
		10.00	1
		7.40	1
	V.vinifera	2.00	0
		4.50	1
		0.17	0

Trigonalyid type	Leaf species	Residency time	Oviposition Pres/Abs
cont.	cont.	7.00	0
		1.00	0
		10.00	0
		2.00	0
Naive fed	E.camal'sis	3.00	0
		5.00	0
		0.33	0
		0.02	0
		0.02	0
		1.00	0
		0.02	0
		10.00	0
		2.00	0
		0.02	0
		4.00	0
		3.00	0
		3.00	0
		10.00	1
		8.00	0
		3.00	0
		10.00	0
		10.00	1
		1.00	0
		10.00	1
		2.00	1
		4.00	1
		4.00	0
	A.piquata	6.50	1
		0.02	0
		0.37	0
		0.22	0
		0.80	0
		10.00	1
		10.00	1
		5.00	1
		0.17	0
		5.00	1
		10.00	1
		0.17	0
		5.00	1
		8.58	1
	V.vinifera	2.00	1
		10.00	0
		1.00	1
		2.00	0
		2.00	0
		3.00	0
		2.00	0
		1.00	0
		1.00	0
		9.00	1

A 4.6.2

ANOVA table for 1-factor analysis of variance of the influence of trigonalyid type (experienced on Acacia, experienced on E. camaldulensis and naïve fed) on residency time of trigonalyids on E. camaldulensis.

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Between gps	2	49.5	24.8	1.99	0.15
Within gps	52	645.9	12.4		
Total	54	695.4			

A 4.6.3

ANOVA table for 2-factor analysis of variance of trigonalyid type and leaf type on the residency time of 2 types of T. venatoria on the leaves of 3 different plant species. The treatments with T. venatoria experienced on Acacia have been omitted.

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Trig type; NF/E	1	3.9	3.9	0.32	0.57
Leaf species*	2	27.4	13.7	1.14	0.33
Intereaction	2	28.9	14.5	1.21	0.31
Error	79	948.0	12.0		

* See section 5.5.1.3.

A 4.6.4

Three-dimensional contingency table analysis to test the hypothesis that trigonalyid type, leaf species and presence or absence of oviposition are mutually independent.

OBSERVED

	Oviposition present		Oviposition absent		Row Totals
Leaf type	Exper'd (E.c.) trig	Naïve fed trig	Exper'd (E.c.) trig	Naïve fed trig	
E. camaldulensis	8	5	7	18	R1 = 38
A. piquata	7	8	9	6	R2 = 30
V. vinifera	1	3	6	8	R3 = 17

Table Totals T1 = 32 (37.65%) T2 = 53 (62.35%)
Column Totals C1 = 38 (44.71%) C2 = 47 (55.29%) n =85

EXPECTED

	Oviposition present		Oviposition absent		Row Totals
Leaf type	Exper'd (E.c.) trig	Naïve fed trig	Exper'd (E.c.) trig	Naïve fed trig	
E. camaldulensis	6.40	7.91	10.59	13.10	R1 = 38
A. piquata	5.05	6.25	8.36	10.34	R2 = 30
V. vinifera	2.86	3.54	4.74	5.86	R3 = 17

Table Totals T1 = 32 (37.65%) T2 = 53 (62.35%)
Column Totals C1 = 38 (44.71%) C2 = 47 (55.29%) n = 85

Chi-square (7df) = 9.47, P = 0.23; Not significant.

APPENDIX 5

RAW DATA AND ANALYSIS, CHAPTER 6.

A 5.1.1

The percentage water content of each soil sample from each replicate of each treatment.
The time at which the the sample was taken is given as days from the start of the experiment.

Time (days)	30 deg Dry			30 deg Wet			25 deg Dry			25 deg Wet			18 deg Dry			18 deg Wet			Field (24 deg) Dry			Field (24 deg) Wet		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3	18.3
12	10.6	12.6	12.0	12.8	13.2	12.8	15.6	14.9	13.0	14.3	14.3	13.2	14.2	14.2	12.6	14.9	14.1	14.6	14.3	14.3	14.2	18.0	17.5	17.3
26	6.1	5.6	8.7	16.2	12.3	17.6	10.8	11.9	11.9	18.1	20.4	20.8	13.2	10.4	13.6	20.6	22.4	20.0	10.9	9.6	5.5	24.8	15.5	26.8
41	1.1	3.7	6.1	12.6	13.8	15.8	11.5	9.2	11.3	14.8	16.8	12.5	11.5	12.3	7.7	21.9	22.4	18.9	11.0	7.1	7.1	18.8	16.7	18.4
56	0.6	1.3	4.1	16.5	16.8	21.1	6.7	6.9	5.9	19.8	18.1	22.6	8.4	10.9	12.3	19.9	21.6	21.3	9.3	8.2	7.3	16.5	14.7	12.2
71	0.5	0.7	1.4	12.3	9.4	14.9	5.3	4.7	4.4	14.4	12.4	11.9	7.7	10.0	11.6	15.7	18.8	20.9	5.5	0.9	2.0	12.8	8.6	2.4
85	0.8	1.0	1.1	13.6	18.1	18.7	4.2	5.8	1.9	21.1	17.0	15.9	5.8	9.7	6.4	17.6	21.9	22.2	2.8	2.1	5.3	11.7	12.5	2.4
99	0.4	0.5	0.5	9.4	11.1	14.3	2.6	1.5	1.6	15.8	13.0	14.7	2.8	7.1	6.4	16.3	17.3	17.6	1.4	1.5	0.6	11.1	4.1	1.0
125	0.3	0.5	0.5	0.8	2.9	1.6	1.4	1.4	0.8	8.5	9.4	9.2	0.8	6.1	5.1	12.1	13.4	13.6	1.1	0.9	1.1	10.7	7.1	2.8
19	•	•	•	23.4	21.0	23.3	•	•	•	27.4	24.7	24.3	•	•	•	23.7	25.2	25.7	•	•	•	•	•	•
51	•	•	•	22.6	21.1	24.5	•	•	•	24.0	21.4	19.8	•	•	•	19.8	26.0	21.5	•	•	•	•	•	•
78	•	•	•	21.0	18.7	22.8	•	•	•	23.3	22.3	19.9	•	•	•	11.7	22.0	24.8	•	•	•	•	•	•

A 5.1.2

ANOVA table for 2-factor analysis of variance of temperature and wet or dry treatment on soil water content

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Dry=1,Wet=2	1	888.2	888.2	109.77	< 0.0001
Temp= 1,2,3,4	3	194.2	64.7	8.00	<0.005
Intereaction	3	94.8	31.6	3.90	0.03
Error	16	129.5	8.1		

A 5.2.1

The temperature, wet or dry treatment, dry weight and percentage water content of each eonymph are given at T=0, 85 and 125 days from the start of the experiment for each replicate of each treatment.

Treatment	Rep	Time (days)	Eonymph % water	Eonymph dry weight	Treatment	Rep	Time (days)	Eonymph % water	Eonymph dry weight	Treatment	Rep	Time (days)	Eonymph % water	Eonymph dry weight
30 deg DRY	1	125	61.1	0.21	18 deg Wet	cont.	125	66.7	0.25	18 deg Dry	cont.	85	68.2	0.21
		125	64.9	0.27			125	66.1	0.20			85	64.8	0.31
		125	52.6	0.18	Field (24) Dry	1	125	65.7	0.24		2	85	63.5	0.27
		125	52.0	0.12			125	65.3	0.25			85	65.6	0.21
		125	63.2	0.07			125	66.0	0.32			85	64.0	0.31
		125	60.7	0.24			125	65.8	0.26			85	66.3	0.30
		125	62.3	0.20			125	66.7	0.15			85	67.4	0.30
		125	58.6	0.24			125	61.0	0.23			85	64.9	0.27
	2	125	50.7	0.33			125	70.1	0.20			85	67.5	0.25
		125	60.5	0.15			125	72.1	0.24		3	85	65.8	0.25
		125	61.9	0.24			125	66.2	0.23			85	66.3	0.33
		125	55.6	0.16		2	125	60.3	0.29			85	71.7	0.17
		125	59.5	0.17			125	47.2	0.28			85	66.7	0.27
		125	61.8	0.21			125	63.9	0.30			85	65.0	0.28
		125	57.7	0.30			125	52.7	0.26			85	64.2	0.29
		125	61.3	0.29			125	61.6	0.33			85	65.3	0.26
		125	45.9	0.33			125	54.1	0.28			85	66.2	0.23
		125	58.1	0.18			125	62.9	0.36			85	63.9	0.30
		125	55.7	0.39		3	125	63.5	0.27			85	62.5	0.33
		125	50.7	0.37			125	67.2	0.20	18 deg Wet	1	85	63.9	0.35
		125	63.2	0.32			125	71.8	0.22			85	69.5	0.26
		125	60.7	0.24			125	58.1	0.31			85	69.4	0.19
		125	60.0	0.24			125	63.3	0.33			85	69.9	0.31
		125	61.9	0.16			125	59.8	0.35			85	60.6	0.24
		125	57.6	0.14			125	54.1	0.17			85	62.7	0.25
		125	58.6	0.24			125	71.8	0.11			85	66.1	0.20
		125	52.2	0.32	Field (24) Wet	1	125	65.0	0.36		2	85	64.3	0.30
	3	125	50.0	0.25			125	64.1	0.33			85	64.1	0.23
		125	59.2	0.29			125	66.7	0.21			85	64.4	0.31
		125	60.3	0.27			125	66.7	0.28			85	68.4	0.25
		125	55.4	0.29			125	68.1	0.29			85	69.9	0.22
		125	49.3	0.34			125	65.7	0.34		3	85	67.0	0.35
		125	60.0	0.34			125	68.4	0.24			85	68.9	0.14
		125	65.0	0.21			125	70.3	0.19			85	64.7	0.30
		125	55.2	0.30			125	66.2	0.26			85	64.0	0.32
		125	56.9	0.25			125	64.1	0.33			85	65.1	0.37
		125	50.0	0.24			125	75.6	0.10			85	65.8	0.26
		125	51.5	0.32			125	64.0	0.32			85	70.4	0.16
		125	51.9	0.25			125	67.5	0.26			85	66.2	0.24
		125	56.2	0.35			125	69.8	0.32			85	66.7	0.17
		125	45.6	0.37			125	66.2	0.26	Field (24) Dry	1	85	58.3	0.25
		125	55.6	0.28			125	68.4	0.31			85	61.4	0.34
		125	55.3	0.34			125	64.8	0.31			85	60.6	0.26
		125	56.8	0.16			125	64.0	0.32			85	64.2	0.24
		125	50.0	0.17			125	66.2	0.27			85	66.2	0.23
		125	58.6	0.41			125	65.9	0.29			85	61.8	0.26
		125	61.5	0.30			125	66.7	0.39			85	66.2	0.23
30 deg Wet	1	125	63.3	0.26		2	125	66.2	0.24			85	61.6	0.33
		125	62.1	0.25			125	67.2	0.22			85	60.4	0.21
		125	64.4	0.21			125	67.6	0.23			85	63.5	0.27
		125	63.0	0.30			125	66.0	0.32		2	85	63.6	0.36
		125	62.5	0.33			125	64.0	0.27			85	67.0	0.31
		125	67.3	0.16			125	70.2	0.17			85	64.1	0.33
		125	65.8	0.27			125	65.0	0.28			85	63.1	0.24
		125	58.9	0.23		3	125	65.2	0.23			85	62.2	0.31
		125	43.6	0.22	"	"	"	"	"		3	85	64.2	0.29
		125	60.3	0.29	30 deg Dry	1	85	63.6	0.12			85	63.8	0.25
	2	125	65.9	0.30			85	63.8	0.21			85	71.1	0.13
		125	64.7	0.24			85	64.6	0.34			85	61.1	0.28
		125	63.4	0.30			85	70.2	0.14	Field (24) Wet	1	85	68.1	0.23
		125	67.7	0.21			85	69.2	0.28			85	64.4	0.31

			125	48.1	0.28				85	65.7	0.35				85	65.4	0.28
			125	63.8	0.25				85	70.0	0.21				85	66.3	0.27
			125	63.9	0.26				85	67.1	0.24				85	66.0	0.32
			125	65.1	0.36				85	62.5	0.42				85	63.4	0.34
			125	66.2	0.22				85	64.7	0.30			2	85	65.2	0.24
		3	125	*	*				85	65.0	0.28				85	62.4	0.35
	25 deg Dry	1	125	60.3	0.31				85	68.9	0.14				85	63.2	0.39
			125	64.2	0.24			2	85	63.9	0.35				85	58.8	0.21
			125	89.5	0.12				85	65.8	0.26				85	61.4	0.39
			125	63.9	0.26				85	67.3	0.16				85	64.6	0.35
			125	64.7	0.24				85	64.9	0.33				85	62.1	0.33
			125	63.4	0.30				85	66.7	0.23				85	62.9	0.39
			125	62.9	0.26				85	64.6	0.28				85	70.4	0.21
			125	66.2	0.24				85	66.7	0.28				85	64.6	0.23
			125	62.1	0.25				85	64.7	0.18			3	85	66.7	0.30
			125	63.1	0.24				85	66.7	0.25				85	68.7	0.26
			125	65.6	0.22			3	85	70.6	0.23				85	67.1	0.23
			125	63.2	0.25				85	70.8	0.26				85	66.7	0.28
			125	62.7	0.28				85	68.5	0.28				85	65.2	0.31
			125	63.6	0.20				85	66.3	0.28				85	63.7	0.32
			125	87.5	0.03				85	68.3	0.33				85	65.6	0.31
			125	68.3	0.20				85	73.3	0.20	*	*	*	*	*	*
		2	125	64.2	0.19				85	71.6	0.23	30 deg Dry	1	0	67.8	0.29	
			125	74.5	0.24				85	72.0	0.26		2	0	72.2	0.20	
			125	60.2	0.33				85	68.7	0.31		1	0	67.9	0.34	
			125	64.3	0.25	30 deg Wet	1		85	72.9	0.15		2	0	77.6	0.15	
			125	66.7	0.18				85	71.4	0.20		1	0	69.9	0.25	
			125	58.0	0.21				85	78.8	0.11		2	0	69.0	0.26	
			125	62.9	0.36				85	76.4	0.17	30 deg Wet	1	0	70.7	0.24	
			125	36.4	0.28				85	74.2	0.17		2	0	69.2	0.28	
			125	62.0	0.35			2	85	76.7	0.14		1	0	76.5	0.12	
			125	61.4	0.27				85	67.9	0.25		2	0	67.1	0.26	
			125	60.4	0.19				85	70.4	0.24		1	0	68.0	0.33	
			125	74.2	0.23				85	70.9	0.23		2	0	67.4	0.31	
			125	62.5	0.27				85	72.8	0.22	25 deg Dry	1	0	69.0	0.26	
			125	61.1	0.21				85	82.1	0.19		2	0	73.6	0.14	
			125	66.0	0.33				85	76.1	0.21		1	0	70.0	0.21	
			125	69.0	0.13			3	85	67.0	0.30		2	0	74.5	0.14	
			125	62.7	0.28				85	72.5	0.22		1	0	71.8	0.20	
			125	62.1	0.39				85	81.9	0.13		2	0	70.8	0.14	
			125	62.5	0.30				85	78.6	0.24	25 deg Wet	1	0	74.6	0.15	
			125	64.4	0.21				85	73.5	0.13		2	0	66.3	0.31	
			125	61.0	0.32				85	70.0	0.21		1	0	67.1	0.25	
			125	54.5	0.25	25 deg Dry	1		85	70.3	0.22		2	0	64.5	0.33	
			125	63.2	0.28				85	67.6	0.36		1	0	68.3	0.26	
		3	125	63.2	0.21				85	69.7	0.27		2	0	68.5	0.17	
			125	60.7	0.24				85	70.9	0.23	18 deg Dry	1	0	72.1	0.19	
			125	53.7	0.25				85	68.3	0.26		2	0	67.5	0.37	
			125	66.0	0.18				85	66.3	0.28		1	0	67.4	0.29	
			125	63.3	0.18				85	64.7	0.14		2	0	65.9	0.29	
			125	60.3	0.27				85	69.2	0.28		1	0	65.6	0.22	
			125	66.0	0.16				85	71.9	0.16		2	0	70.9	0.16	
			125	62.2	0.34				85	72.3	0.18	18 deg Wet	1	0	66.3	0.27	
			125	57.1	0.30				85	69.7	0.30		2	0	70.2	0.17	
			125	63.6	0.20				85	67.6	0.24		1	0	73.5	0.13	
			125	62.2	0.17			2	85	63.8	0.25		2	0	75.6	0.42	
			125	60.8	0.29				85	69.2	0.28		1	0	69.8	0.19	
			125	64.5	0.22				85	71.2	0.21		2	0	67.0	0.35	
			125	69.0	0.18				85	70.0	0.27	Field (24) Dry	1	0	65.9	0.30	
	25 deg Wet	1	125	65.8	0.27				85	68.1	0.30		2	0	70.2	0.14	
			125	65.3	0.25				85	71.4	0.26		1	0	65.6	0.32	
			125	62.2	0.31				85	72.1	0.19		2	0	72.2	0.15	
		2	125	66.0	0.35				85	65.2	0.31		1	0	68.5	0.29	
			125	66.3	0.34				85	70.6	0.20		2	0	62.9	0.33	
			125	63.5	0.35				85	67.3	0.33	Field (24) Wet	1	0	66.7	0.26	
			125	64.0	0.31				85	73.4	0.27		2	0	70.7	0.17	
			125	65.0	0.35				85	68.8	0.25		1	0	75.7	0.09	
			125	64.2	0.34				85	70.4	0.24		2	0	68.9	0.32	
			125	60.0	0.28				85	68.1	0.29		1	0	67.1	0.23	
			125	63.0	0.27			3	85	74.6	0.15		2	0	68.1	0.22	
			125	59.5	0.32				85	67.0	0.33						
			125	64.9	0.33				85	69.3	0.27						
			125	61.8	0.34				85	71.2	0.19						
			125	62.8	0.29				85	76.1	0.11						
			125	64.3	0.30				85	75.0	0.15						
			125	63.5	0.19				85	81.2	0.13						
			125	62.0	0.30				85	73.8	0.17						
			125	61.3	0.29				85	67.0	0.34						

		125	66.7	0.16			85	66.3	0.33
		125	65.9	0.29			85	68.4	0.25
		125	69.1	0.17			85	67.4	0.29
		125	64.3	0.25	25 deg Wet	1	85	80.3	0.13
	3	125	66.7	0.21			85	66.0	0.33
		125	67.6	0.23			85	68.1	0.30
		125	62.9	0.26			85	78.8	0.11
		125	66.2	0.27			85	68.1	0.25
		125	63.5	0.27			85	68.9	0.28
		125	65.0	0.28			85	68.8	0.25
		125	66.0	0.33			85	65.2	0.31
18 deg Dry	1	125	69.4	0.19		2	85	65.7	0.36
		125	50.9	0.27			85	68.0	0.32
	2	125	61.9	0.32			85	67.9	0.26
		125	66.2	0.24			85	73.6	0.14
		125	64.9	0.34			85	69.5	0.25
		125	61.6	0.38			85	73.9	0.18
		125	67.8	0.28			85	77.8	0.20
		125	67.1	0.24			85	69.6	0.24
		125	65.1	0.29			85	69.6	0.28
		125	67.5	0.27			85	70.1	0.28
		125	65.3	0.25			85	76.3	0.18
		125	61.8	0.29		3	85	74.1	0.15
		125	65.4	0.27			85	74.0	0.19
	3	125	66.2	0.22			85	72.1	0.19
		125	63.5	0.31			85	74.3	0.19
		125	63.9	0.26			85	69.5	0.29
		125	63.6	0.32			85	67.8	0.29
		125	66.7	0.18			85	70.5	0.26
		125	67.2	0.21			85	72.9	0.19
		125	65.1	0.29			85	67.0	0.36
		125	64.0	0.27			85	67.5	0.26
18 deg Wet	1	125	63.3	0.33			85	73.2	0.15
		125	64.0	0.32			85	72.0	0.21
		125	75.6	0.22			85	66.0	0.34
		125	61.5	0.27	18 deg Dry	1	85	64.9	0.26
		125	62.5	0.30			85	63.0	0.27
		125	59.7	0.27			85	67.9	0.18
		125	66.7	0.24			85	70.0	0.09
	2	125	65.9	0.28			85	62.8	0.16
		125	62.2	0.31			85	66.7	0.28
	3	125	64.3	0.25			85	71.1	0.11
		125	62.0	0.27			85	68.3	0.20
		125	66.1	0.21					
		125	66.7	0.28					

A 5.2.2 (a)

ANOVA table for 2-factor analysis of variance of temperature and wet or dry treatment on the dry weight of eonymphs at 85 days from the start of the experiment.

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Dry=1,Wet=2	1	0.00	0.00	0.558	0.456
Temp=1,2,3,4	3	0.08	0.03	6.389	0.0004
Intereaction	3	0.05	0.02	4.328	0.0056
Error	200	0.80	0.00		

A 5.2.2 (b)

ANOVA table for 2-factor analysis of variance of temperature and wet or dry treatment on the percent water content of eonymphs at 85 days from the start of the experiment.

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Dry=1,Wet=2	1	280.10	280.10	27.195	0.0001
Temp=1,2,3,4	3	1473.61	491.20	47.692	0.0001
Intereaction	3	326.57	108.86	10.569	0.0001
Error	200	2059.90	10.30		

A 5.2.3

Fisher PLSD between mean eonymph percent water content at 85 days for 4 different temperatures (30, 25, 18, and 24 deg. C)

Temp. (deg. C)	Mean difference	Fisher PLSD
30 vs 25	-0.51	1.3281
30 vs 18	3.63	1.4466*
30 vs 24	5.59	1.4974*
25 vs 18	4.14	1.3281*
25 vs 24	6.10	1.3833*
18 vs 24	1.96	1.4974*

* Significant at 95% level.

A 5.3.1

ANOVA table for 1-factor analysis of variance of time (T=0, 85 and 125 days) on the dry weight of eonymphs, and the Fisher PLSD between time groups.

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Between gps	2	0.0	0.0	2.82	0.06
Within gps	491	2.1	0.0		
Total	493	2.1			

Time gp (days)	Mean difference	Fisher PLSD
0 vs 85	-0.01	0.021
0 vs 125	-0.02	.02*
85 vs125	-0.01	0.012

* Sig at 95% level

A 5.3.2

ANOVA table for 1-factor analysis of variance of time (T=0, 85 and 125 days) on the percent water content of eonymphs, and the Fisher PLSD between time groups.

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Between gps	2	3872.0	1936.0	73.15	0.0001
Within gps	491	12995.8	26.5		
Total	493	16867.9			

Time gp (days)	Mean difference	Fisher PLSD
0 vs 85	1.50	1.619
0 vs 125	6.76	1.6*
85 vs125	5.26	.96*

* Sig at 95% level

A 5.4.1

The pecentage emergence and percentage mortality for each replicate (pot) in each treatment. The mean percentage emergence and mortality are also given for each treatment.

Treatment	Replicate	N 1*	% Emergence	Treat mean	N 2*	% Mortality	Treat Mean
30 deg Dry	1	12	25.0		49	69.4	
	2	25	4.0		52	44.2	
	3	27	7.4	12.1	52	17.3	43.6
30 deg Wet	1	21	30.4		56	53.6	
	2	36	12.5		54	66.7	
	3	27	0.0	14.3	55	94.6	71.6
25 deg Dry	1	23	34.5		49	44.9	
	2	16	27.5		47	27.7	
	3	2	34.4	32.1	48	35.4	36.0
25 deg Wet	1	20	63.2		54	66.7	
	2	11	37.0		50	42.0	
	3	12	25.0	41.7	48	68.8	59.2
18 deg Dry	1	29	81.0		51	41.2	
	2	40	58.3		54	40.7	
	3	32	51.8	63.7	51	56.9	46.3
18 deg Wet	1	23	60.0		54	63.0	
	2	13	81.8		56	73.2	
	3	13	50.0	63.9	52	76.9	71.0
Field (24) Dry	1	19	39.1		51	66.7	
	2	54	38.5		56	69.6	
	3	12	0.0	25.9	57	73.7	68.5
Field (24) Wet	1	31	29.0		55	50.9	
	2	17	35.3		51	80.4	
	3	7	71.4	45.2	54	81.5	70.9

N1* = Number of potential emergences (Number emerged + Number in prolonged diapause)
N2* = Number of cocoons in replicate at 125 days (after subsamplig eonymphs at 0 and 85 days).

A 5.4.2 (a)

ANOVA table for 2-factor analysis of variance of temperature and wet or dry treatment on the percent emergence of eonymphs.

Source	df:	Sum of Squares:	Mean Square:	F - test:	P value:
Dry=1,Wet=2	1	369.8	369.8	1.30	0.27
Temp=1,2,3,4	3	7718.5	2572.8	9.02	<0.001
Intereaction	3	339.7	113.2	0.40	0.76
Error	16	4562.7	285.2		

A 5.4.2 (b)

Fisher PLSD between mean percent emergence for 4 different temperatures (30, 25, 18, and 24 deg. C).

Temp. (deg. C)	Mean difference	Fisher PLSD
30 vs 25	-23.70	19.5558*
30 vs 18	-50.61	19.5558*
30 vs 24	-22.33	19.5558*
25 vs 18	-26.90	19.5558*
25 vs 24	1.37	19.5558
18 vs 24	28.27	19.5558*

* Significant at 95% level.

A 5.4.3

The soil percentage water content, mean eonymph water content and mean eonymph dry weight for each replicate (pot) at 85 days, and the corresponding percent emergence and percent mortality.

Treatment	Rep	Soil water on day 85 (%)	Mean eonymph % H20 T=85	Mean eonymph dry wt T=85	% emergence	% mortality
30 deg Dry	1	0.80	66.28	0.25	25.00	73.30
	2	1.00	65.70	0.26	4.00	43.20
	3	1.10	70.01	0.26	7.40	18.20
30 deg Wet	1	13.60	74.74	0.16	30.40	53.10
	2	18.10	73.84	0.21	12.50	67.40
	3	18.70	73.92	0.21	0.00	93.90
25 deg Dry	1	4.20	69.04	0.24	34.50	44.20
	2	5.80	69.19	0.26	27.50	27.30
	3	1.90	71.44	0.23	34.40	36.00
25 deg Wet	1	21.10	70.65	0.25	63.20	67.20
	2	17.00	71.09	0.25	37.00	42.60
	3	15.90	70.84	0.26	25.00	69.20
18 deg Dry	1	5.80	66.09	0.21	81.00	41.80
	2	9.70	65.60	0.27	58.30	41.40
	3	6.20	65.76	0.27	51.90	56.10
18 deg Wet	1	17.60	66.01	0.26	60.00	62.30
	2	21.90	66.22	0.26	81.80	73.20
	3	22.20	66.53	0.26	50.00	76.90
Field (24) Dry	1	2.80	62.42	0.26	39.10	66.20
	2	2.10	64.00	0.31	38.50	69.00
	3	5.30	65.05	0.27	0.00	73.50
Field (24) Wet	1	11.70	65.60	0.29	29.00	51.60
	2	12.50	63.56	0.31	35.30	81.30
	3	2.40	66.24	0.29	71.40	82.10

A 5.4.4

Simple regression of percent emergence from each pot (replicate) on soil percent water content.

df	R	R-squared	Adj. R-squared	S.E.
23	0.221	0.049	0.005488	23.701

ANOVA table

Source	df	Sum squares	Mean square	F-test
Regression	1	633.0	633.0	1.13
Residual	22	12357.7	561.7	**p = 0.30**
Total	23	12990.7		

A 5.4.5

Simple regression of percent emergence from each pot (replicate) on the average eonymph percent water content for each pot.

df	R	R-squared	Adj. R-squared	S.E.
23	0.324	0.105	0.064	22.989

ANOVA table

Source	df	Sum squares	Mean square	F-test
Regression	1	1364.2	1364.2	2.58
Residual	22	11626.5	528.5	**p = 0.12**
Total	23	12990.7		

A 5.4.6

Simple regression of percent emergence from each pot (replicate) on the mean dry weight of eonymphs in each pot.

df	R	R-squared	Adj. R-squared	S.E.
23	0.106	0.011	-0.034	24.136

ANOVA table

Source	df	Sum squares	Mean square	F-test
Regression	1	146.0	146.0	0.25
Residual	22	12844.7	583.9	**p = 0.62**
Total	23	12990.7		

A 5.5

ANOVA table for 2-factor analysis of variance of temperature and wet or dry treatment on the mortality in each pot (replicate).

Source	df:	Sum of Squares	Mean Square:	F - test:	P value:
Dry=1,Wet=2	1	2215.7	2215.7	9.21	0.01
Temp= 1,2,3,4	3	1572.9	524.3	2.18	0.13
Intereaction	3	592.3	197.4	0.82	0.50
Error	16	3848.1	240.5		

A 5.6

Simple regression of percent mortality in each pot (replicate) on the average dry weight of eonymphs in each pot at T=85.

df	R	R-squared	Adj. R-squared	S.E.
23	0.131	0.017	-0.027	19.173

ANOVA table

Source	df	Sum squares	Mean square	F-test
Regression	1	141.6	141.6	0.39
Residual	22	8087.3	367.6	**p = 0.54**
Total	23	8230.0		

APPENDIX 6

RAW DATA, CHAPTER 8.

Appendix 6

The weight (g) of each larva in 4 matched pairs of treatment (even colony numbers) and control (odd colony numbers), after 9 weeks of feeding.

Larva number	Colony number							
	1	2	3	4	5	6	7	8
1	1.50	1.93	1.50	1.08	0.66	0.68	0.59	1.30
2	1.88	0.76	0.90	1.78	0.74	0.56	0.81	1.12
3	1.45	1.45	1.34	2.01	0.66	0.79	0.89	0.88
4	2.19	1.82	0.80	2.00	0.70	0.68	0.54	1.19
5	1.31	2.81	1.67	2.03	0.90		0.32	1.34
6	1.30	1.65	0.65	2.27	0.79		0.58	1.22
7	1.24	1.25	1.07	1.60	0.65		1.20	1.03
8	1.74	1.53	1.11	2.08	0.66		1.04	0.88
9	1.92	1.18	1.67	1.25	0.45		0.59	0.73
10	1.33	0.81	1.25	1.26	0.65		0.67	1.26
11	1.20	1.37	1.66	2.39	0.81		0.57	1.10
12	2.02	1.69	1.84	2.44	0.38		0.67	1.24
13	1.19	2.65	2.31	0.90	0.69		1.05	1.49
14	1.02	1.59	2.50	1.33	0.62		0.88	0.84
15	1.66	1.67	1.67	2.45	0.36		1.05	0.66
16	1.97	2.06	0.66	1.31	0.55		0.69	1.50
17	0.57	1.12	2.23	0.94	0.65		1.12	0.83
18	2.01	1.94	0.76	2.44	0.35		0.75	1.32
19	1.36	1.70	0.87	1.93	0.50		0.90	0.66
20	0.49	1.92	2.33	0.89	0.70		0.64	0.88
21	0.70	2.10	1.08	1.68	0.66		0.89	1.07
22	1.28	1.19	0.91	1.85	0.69		0.45	0.97
23	1.16	1.45	0.68	1.35	0.68		0.43	0.85
24	1.86	1.82	1.64	0.69	0.40		0.28	1.35
25	1.98	1.22	0.81	1.49	0.62		0.87	0.91
26	1.27	0.90	0.97	1.05	0.84		0.81	0.91
27	1.18	1.05	0.61	1.66	0.87		0.57	0.67
28	1.86	1.96	0.59	0.72			0.88	1.29
29	0.63	2.26	0.12	0.66			0.56	1.10
30	1.97	2.53	0.88	1.95			0.59	1.36
31	0.88	1.11	1.65	2.04			0.77	0.74
32	1.22	1.21	2.33	0.63			0.54	1.33
33	0.71	1.78	1.61	0.28			0.54	1.03
34	1.60	1.40	2.55	0.74			0.94	0.98
35	2.21	2.00	1.24	1.47			0.67	1.48
36	1.74	1.76	2.53	1.55			1.04	1.59
37	1.50	1.31	1.78	2.22			0.48	1.18
38	1.23	1.26	0.92	0.82				0.93
39	1.93	1.06	1.83	1.38				1.24
40	1.14	1.11	2.04	0.83				1.27
41	0.77	2.08	1.72	1.05				1.41
42	1.79	1.48	0.58	1.73				1.42
43	0.76	1.56	1.66	1.82				1.03
44		1.90	1.95	1.21				1.23
45			0.83	1.45				0.44
46			0.81	0.62				0.92
47			0.74	1.08				1.27
48			0.95	0.45				1.17
49			0.37	0.89				1.00
50			0.73	0.82				1.20
51			1.65	0.43				1.36
52			2.00	0.95				0.70
53			0.93	0.42				1.47
54			1.27	1.37				0.38
55			2.48	0.34				0.70
56			0.83	0.49				0.77
57			1.29	0.77				0.84
58			0.79	0.67				0.77
59			1.03					0.96
60			1.64					1.15
61			0.47					
62			0.75					
63			0.44					
64			1.07					

Larva number	Colony number							
	1	2	3	4	5	6	7	8
65			0.64					
66			0.96					
67			0.42					
68			0.49					
69			1.92					
70			0.59					
71			0.82					
72			0.54					
73			1.29					
74			1.67					
75			0.76					
76			1.01					
78			0.77					
79			0.77					
80			1.54					
81			2.01					
82			1.93					
83			1.10					
84			0.72					
85			0.21					
86			1.18					
87			0.64					
88			0.48					

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